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(54) **ISOLATED NUCLEIC ACID MOLECULE(S) ENCODING A HUMAN CALCIUM SENSITIVE POTASSIUM CHANNEL SUBUNIT PROTEIN DESIGNATED BETA2, ENCODED PROTEINS, AND USES THEREOF**

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(57) **ABSTRACT**

The present invention is directed to novel human DNA sequences encoding calcium sensitive potassium channel subunits β2, β3a, β3b, β3c, and β3d, the proteins encoded by the DNA sequences, vectors comprising the DNA sequences, host cells containing the vectors, and methods of identifying inhibitors and agonists of calcium sensitive potassium channels containing human β2, β3a, β3b, β3c, or β3d subunits and inhibitors and agonists of β3 gene transcription.

```
   1  CTTAATCCTA TCCAAGTATG CAGTACGCTC TTGGGTCGTC TCATGAGACC CAGGGGCATG
  61  TTGGAAAGAA CTGAGAGAAA GAGCAACAAA GCGGCGAGTG GTGTGAGAGG GCAGCACGCG
 121  CTGTGGGGCC CTTCCAGAGA AATGTACTGA AAAAGTCTAC GCAATGTCTG GGATTTGCTA
 181  AACAATACCT GGAAAGCAGA CAGGTTTTTT TGCCATTCCT CCAGGACATC CACCATAAGG
 241  AAAGGAGACC CTGGACCAAC ATTCTCTAAG ATGTTTATAT GGACCAGTGG CCGGACCTCT
 301  TCATCTTATA GACATGATGA AAAAAGAAAT ATTTACCAGA AAATCAGGGA CCATGACCTC
 361  CTGGACAAAA GGAAAACAGT CACAGCACTG AAGGCAGGAG AGGACCGAGC TATTCTCCTG
 421  GGACTGGCTA TGATGGTGTG CTCCATCATG ATGTATTTTC TGCTGGGAAT CACACTCCTG
 481  CGCTCATACA TGCAGAGCGT GTGGACCGAA GAGTCTCAAT GCACCTTGCT GAATGCGTCC
 541  ATCACGGAAA CATTTAACTG CTCCTTCAGC TGTGGTCCAG ACTGCTGGAA ACTTTCTCAG
 601  TACCCCTGCC TCCAGGTGTA CGTTAACCTG ACTTCTTCCG GGGAAAAGCT CCTCCTCTAC
 661  CACACAGAAG AGACAATAAA AATCAATCAG AAGTGCTCCT ATATACCTAA ATGTGGAAAA
 721  AATTTTGAAG AATCCATGTC CCTGGTGAAT GTTGTCATGG AAAACTTCAG GAAGTATCAA
 781  CACTTCTCCT GCTATTCTGA CCCAGAAGGA AACCAGAAGA GTGTTATCCT AACCAAACTC
 841  TACAGTTCCA ACGTGCTGTT CCATTCACTC TTCTGGCCAA CCTGTATGAT GGCTGGGGGT
 901  GTGGCAATTG TTGCCATGGT GAAACTTACA CAGTACCTCT CCCTACTATG TGAGAGGATC
 961  CAACGGATCA ATAGATAAAT GCAAAAATGG ATAAAATAAT TTTTGTTAAA GCTCAAATAC
1021  TGTTTTCTTT CATTCTTCAC CAAAGAACCT TAAGTTTGTA ACGTGCAGTC TGTTATGAGT
1081  TCCCTAATAT ATTCTTATAT GTAGAGCAAT AATGCAAAAG CTGTTCTATA TGCAAACATG
1141  ATGTCTTTAT TATTCAGGAG AATAAATAAC TGTTTTGTGT TGAA
```

FIG.1A

```
   1  MFIWTSGRTS SSYRHDEKRN IYQKIRDHDL LDKRKTVTAL KAGEDRAILL GLAMMVCSIM
  61  MYFLLGITLL RSYMQSVWTE ESQCTLLNAS ITETFNCSFS CGPDCWKLSQ YPCLQVYVNL
 121  TSSGEKLLLY HTEETIKINQ KCSYIPKCGK NFEESMSLVN VVMENFRKYQ HFSCYSDPEG
 181  NQKSVILTKL YSSNVLFHSL FWPTCMMAGG VAIVAMVKLT QYLSLLCERI QRINR
```

FIG.1B

```
   1 GCTCCCGGCT GCCGAGGCGG AAACACAGGT GATGAGGTGG CGGCAAGCAC AGTGCAAAGA
  61 GAGAGAAGCA GCTTCGGCTG CAGCAAACCA CGCAGGTCCT TCTTGATCAT CTAGAACTGA
 121 CCGCTCCGCC TTGCCAGGAG TCTGCAGAAC CACGTGGCTG GCCTGCCTGA AGTTCTCACC
 181 TCTCTAGGAA GGCGGGGGGC TTCTAATGGC TGCAGCTGCG CTGGGGGCTG GGGGCTCCCG
 241 CTGGGACTCC ACTTCCGTGG ATGTCTAAGC TTCACCTTTC TTGCGCCCGC AGGGGCATGA
 301 CTCAGGTGAA AGGGAGCCAT TTTCTCAGAC CCCTGGCCTC ATGCAGCCCT TCAGCATCCC
 361 CGTGCAAATC ACACTTCAGG GCAGCCGGAG GCGCCAGGGG AGGACAGCCT TTCCTGCCTC
 421 AGGGAAGAAG AGAGAGACAG ACTACAGTGA TGGAGACCCA CTAGATGTGC ACAAGAGGCT
 481 GCCATCCAGT ACTGGAGAGG ACCGAGCCGT GATGCTGGGG TTTGCCATGA TGGGCTTCTC
 541 AGTCCTAATG TTCTTCTTGC TCGGAACAAC CATTCTAAAG CCTTTTATGC TCAGCATTCA
 601 GAGAGAAGAA TCGACCTGCA CTGCCATCCA CACAGATATC ATGGACGACT GGCTGGACTG
 661 TGCCTTCACC TGTGGTGTGC ACTGCCACGG TCAGGGGAAG TACCCGTGTC TTCAGGTGTT
 721 TGTGAACCTC AGCCATCCAG GTCAGAAAGC TCTCCTACAT TATAATGAAG AGGCTGTCCA
 781 GATAAATCCC AAGTGCTTTT ACACACCTAA GTGCCACCAA GATAGAAGTG ATTTGCTCAA
 841 CAGTGCTCTG GACATAAAAG AATTCTTCGA TCACAAAAAT GGAACCCCCT TTTCATGCTT
 901 CTACAGTCCA GCCAGCCAAT CTGAAGATGT CATTCTTATA AAAAAGTATG ACCAAATGGC
 961 TATCTTCCAC TGTTTATTTT GGCCTTCACT GACTCTGCTA GGTGGTGCCC TGATTGTTGG
1021 CATGGTGAGA TTAACACAAC ACCTGTCCTT ACTGTGTGAA AAATATAGCA CTGTAGTCAG
1081 AGATGAGGTA GGTGGAAAAG TACCTTATAT AGAACAGCAT CAGTTCAAAC TGTGCATTAT
1141 GAGGAGGAGC AAAGGAAGAG CAGAGAAATC TTAAGACGGT GGCCAAATTA AAGTGCTGGC
1201 CTTCAGATGT CTGTGATTTC TGCAACTCGA GTATGCG
```

FIG. 2A

```
   1 MQPFSIPVQI TLQGSRRRQG RTAFPASGKK RETDYSDGDP LDVHKRLPSS TGEDRAVMLG
  61 FAMMGFSVLM FFLLGTTILK PFMLSIQREE STCTAIHTDI MDDWLDCAFT CGVHCHGQGK
 121 YPCLQVFVNL SHPGQKALLH YNEEAVQINP KCFYTPKCHQ DRSDLLNSAL DIKEFFDHKN
 181 GTPFSCFYSP ASQSEDVILI KKYDQMAIFH CLFWPSLTLL GGALIVGMVR LTQHLSLLCE
 241 KYSTVVRDEV GGKVPYIEQH QFKLCIMRRS KGRAEKS
```

FIG. 2B

```
   1  AAGAGAAAGA ACAAGAAAAA GAAAAAGAAG AGGAAAAAAT CCCCAGTACC CATAGAAACC
  61  CTTAAAGATG TTTAAAAAGA GTTAACTTAT CAGAACACAG ATTTAAGTGA AATTAAGGA
 121  GAAGAGCAGG TAAAGTCTAC TGACAGAAAG TCAGCAGTGG AAGCCCAAAA CGAGGTGACT
 181  GAAAATCCAA AACAGAAAAT TGCAGCAGAA AGCAGTGAAA ATGCTGATTG TCCAGAGAAT
 241  CCTAAAATGA AGTTGGATGG AAAACTTGAC CAAGAAGGCA ATGATGTAAA AACAGCAGCT
 301  GAGGAGGTAC TAGCTGGTAG AGACACATTA GATTTTGAGG ATGTCACAGT TCAATCATCA
 361  GGCCCGAGGG CTGGTGGTGA AGAATTAGAT GAAGGTGTTG CAAAAGATAA TGCTAAAATA
 421  GCTGGTGCCA CTTAAAGCAA TCCTGAAGAA CCAGAGAGTG AAGATGCAGA TCACTGCACC
 481  GTACCCAAAA ATGAAAGTCC CTCACAGGAC ATTAGTGATG CCTGTGAAGC AGAAAGTACA
 541  GAGAGGTGCG GGATGTCAGA ACATCCAAGT CAGACCATCA GGAAAGCTTT AGACAGCAAT
 601  AGCCTAAAAA ACCATGACTT GTTGGCACCA GGAGGAGAGC CGGGGGACTT CAATCCAGAA
 661  AGCAGAGAAG ATACCAGAGG AGGGAACGAG AAGGGCAAAA GCAAAGAAGA CCGTACCATG
 721  TCCTAAGCTG AGGCAGGCGG CAGGCGTGGT GCACAGGAAG TCTGAGTGTG AGGGGCTCTT
 781  TTCTCTCCAC TGCCAATGAC AGCCTTTCCT GCCTCAGGGA AGAAGAGAGA GACAGACTAC
 841  AGTGATGGAG ACCCACTAGA TGTGCACAAG AGGCTGCCAT CCAGTACTGG AGAGGACCGA
 901  GCCGTGATGC TGGGGTTTGC CATGATGGGC TTCTCAGTCC TAATGTTCTT CTTGCTCGGA
 961  ACAACCATTC TAAAGCCTTT TATGCTCAGC ATTCAGAGAG AAGAATCGAC CTGCACTGCC
1021  ATCCACACAG ATATCATGGA CGACTGGCTG GACTGTGCCT TCACCTGTGG TGTGCACTGC
1081  CACGGTCAGG GGAAGTACCC GTGTCTTCAG GTGTTTGTGA ACCTCAGCCA TCCAGGTCAG
1141  AAAGCTCTCC TACATTATAA TGAAGAGGCT GTCCAGATAA ATCCCAAGTG CTTTTACACA
1201  CCTAAGTGCC ACCAAGATAG AAATGATTTG CTCAACAGTG CTCTGGACAT AAAAGAATTC
1261  TTCGATCACA AAAATGGAAC CCCCTTTTCA TGCTTCTACA GTCCAGCCAG CCAATCTGAA
1321  GATGTCATTC TTATAAAAAA GTATGACCAA ATGGCTATCT TCCACTGTTT ATTTTGGCCT
1381  TCACTGACTC TGCTAGGTGG TGCCCTGATT GTTGGCATGG TGAGATTAAC ACAACACCTG
1441  TCCTTACTGT GTGAAAAATA TAGCACTGTA GTCAGAGATG AGGTAGGTGG AAAAGTACCT
1501  TATATAGAAC AGCATCAGTT CAAACTGTGC ATTATGAGGA GGAGCAAAGG AAGAGCAGAG
1561  AAATCTTAAG ACGGTGGCCA AATTAAAGTG CTGGCCTTCA GATGTCTGTG ATTTCTGCAA
1621  CTCGAGTATG CG
```

FIG.3A

```
  1 MTAFPASGKK RETDYSDGDP LDVHKRLPSS TGEDRAVMLG FAMMGFSVLM FFLLGTTILK
 61 PFMLSIQREE STCTAIHTDI MDDWLDCAFT CGVHCHGQGK YPCLQVFVNL SHPGQKALLH
121 YNEEAVQINP KCFYTPKCHQ DRNDLLNSAL DIKEFFDHKN GTPFSCFYSP ASQSEDVILI
181 KKYDQMAIFH CLFWPSLTLL GGALIVGMVR LTQHLSLLCE KYSTVVRDEV GGKVPYIEQH
241 QFKLCIMRRS KGRAEKS
```

FIG.3B

```
   1 CCCAGCTACT CGGGAGGCTG AGGCAGGAGA ATCGCTTGAA CCTGGGAGGC GGAGGAGGTT
  61 GCAGTGAACT GAGATCGTAC CCAGCCTGGG CAACAGTGCG AGGCTCCGTC TCAAAAAAAA
 121 ACCAAAAAAC ACAAAAACAA AAAACGACAG AGAAGGCCAA ACAAAACACA TCTGTGGGCT
 181 GGATGCCGCC ATGCCCACCG GTTTGCGACC TTTGTGTTGG ACTCTTCTGT TCACCAGACA
 241 CCCTGCCCTG CGAGAATGTA TCTCATCCTT TGCTGGAGCA GGTTTGCAGG CACAGTGGAG
 301 AGAGGAGAGA AGAAATGAAG GGACACTTAT GCAGAACCAT GAGTGGCCAG AGAGGAGGAG
 361 AAGGAGGGTG AGAGGAGCAA AGAAGCCATG ACAACTTCAT AATTCTGAGT GGACTGGGCA
 421 GTGGCCAGAA ATTCTGGTGG TGGATATGCT GCCTTTCCAA CAGGTGAATA TGAAAGAATA
 481 AGTCAAACCC TGTTCAGGAC GCTGTTAATT CCAAATGTGA ACTTTTTGAG TCATTCTTTT
 541 CATGTGGAAT TCAAAGGAGA ATGTAAACAA ATTTTCAGGA GGGACGTGCA ATATCCCTGA
 601 AAGATAACAG AGTTCGTAAC ACTTATTTAC ATACAACATT CTCTAGTTAT TGATTAAACA
 661 GATCTCTACA GACTTGCATG AGGCAACATT TCTTAGGCTT GTTTGCTACA ATATCTTTAA
 721 AAATACTTGA TTACACATCA CTTTAGCTTA TTTAGATGGA CTTTTCACCA AGCTCTGAAC
 781 TGGGATTTCA TTTTGTTGCA TTCATCCTGC TCACGAGACA CAGGTAGGCA GCAAATGAGA
 841 TTATCCCTCC AGTCCCCATG GATTGGAAAT GTTCCCCCTT CTTTATGAGC TCACTGCAGT
 901 ATCTCCTTCT CCCTTTCCCC AAAGGACAGC CTTTCCTGCC TCAGGGAAGA AGAGAGAGAC
 961 AGACTACAGT GATGGAGACC CACTAGATGT GCACAAGAGG CTGCCATCCA GTACTGGAGA
1021 GGACCGAGCC GTGATGCTGG GGTTTGCCAT GATGGGCTTC TCAGTCCTAA TGTTCTTCTT
1081 GCTCGGAACA ACCATTCTAA AGCCTTTTAT GCTCAGCATT CAGAGAGAAG AATCGACCTG
1141 CACTGCCATC CACACAGATA TCATGGACGA CTGGCTGGAC TGTGCCTTCA CCTGTGGTGT
1201 GCACTACCAC GGTCAGGGGA AGTACCCGTG TCTTCAGGTG TTTGTGAACC TCAGCCATCC
1261 AGGTCAGAAA GCTCTCCTAC ATTATAATGA AGAGGCTGTC CAGATAAATC CCAAGTGCTT
1321 TTACACACCT AAGTGCCACC AAGATAGAAG TGATTTGCTC AACAGTGCTC TGGACATAAA
1381 AGAATTCTTC GATCACAAAA ATGGAACCCC CTTTTCATGC TTCTACAGTC CAGCCAGCCA
1441 ATCTGAAGAT GTCATTCTTA TAAAAAAGTA TGACCAAATG GCTATCTTCC ACTGTTTATT
1501 TTGGCCTTCA CTGACTCTGC TAGGTGGTGC CCTGATTGTT GGCATGGTGA GATTAACACA
1561 ACACCTGTCC TTACTGTGTG AAAAATATAG CACTGTAGTC AGAGATGAGG TAGGTGGAAA
1621 AGTACCTTAT ATAGAACAGC ATCAGTTCAA ACTGTGCATT ATGAGGAGGA GCAAAGGAAG
1681 AGCAGAGAAA TCTTAAGACG GTGGCCAAAT TAAAGTGCTG GCCTTCAGAT GTCTGTGATT
1741 TCTGCAACTC GAGTATGCG
```

FIG.4A

```
  1 MFPLLYELTA VSPSPFPQRT AFPASGKKRE TDYSDGDPLD VHKRLPSSTG EDRAVMLGFA
 61 MMGFSVLMFF LLGTTILKPF MLSIQREEST CTAIHTDIMD DWLDCAFTCG VHCHGQGKYP
121 CLQVFVNLSH PGQKALLHYN EEAVQINPKC FYTPKCHQDR SDLLNSALDI KEFFDHKNGT
181 PFSCFYSPAS QSEDVILIKK YDQMAIFHCL FWPSLTLLGG ALIVGMVRLT QHLSLLCEKY
241 STVVRDEVGG KVPYIEQHQF KLCIMRRSKG RAEKS
```

FIG.4B

```
   1 CGCCGCGGAT CCGAAATGAA GGGACACTTA TGCAGAACCA TGAGTGGCCA GAGAGGAGGA
  61 GAAGGAGGGT GAGAGGAGCA AAGAAGCCAT GACAACTTCA TAATTCTGAG TGGACTGGGC
 121 AGTGGCCAGA AATTCTGGTG GTGGATATGC TGCCTTTCCA ACAGGTGAAT ATGAAAGAAT
 181 AAGTCAAACC CTGTTCAGGA CGCTGTTAAT TCCAAATGTG AACTTTTTGA GTCATTCTTT
 241 TCATGTGGAA TTCAAAGGAG AATGTAAACA AATTTTCAGG AGGGACGTGC AATATCCCTG
 301 AAAGATAACA AAGTTCGTAA CACTTATTTA CATACAACAT TCTCTAGTTA TTGATTAAAC
 361 AGATCTCTAC AGACTTGCAT GAGGCAACAT TTCTTAGGCT TGTTTGCTAC AATATCTTTA
 421 AAAATACTTG ATTACACATC ACTTTAGCTT ATTTAGATGG ACTTTTCACC AAGCTCTGAA
 481 CTGGGATTTC ATTTTGTTGC ATTCATCCTG CTCACGAGAC ACAGGACAGC CTTTCCTGCC
 541 TCAGGGAAGA AGAGAGAGAC AGACTACAGT GATGGAGACC CACTAGATGT GCACAAGAGG
 601 CTGCCATCCA GTACTGGAGA GGACCGAGCC GTGATGCTGG GGTTTGCCAT GATGGGCTTC
 661 TCAGTCCTAA TGTTCTTCTT GCTCGGAACA ACCATTCTAA AGCCTTTTAT GCTCAGCATT
 721 CAGAGAGAAG AATCGACCTG CACTGCCATC CACACAGATA TCATGGACGA CTGGCTGGAC
 781 TGTGCCTTCA CCTGTGGTGT GCACTGCCAC GGTCAGGGGA AGTACCCGTG TCTTCAGGTG
 841 TTTGTGAACC TCAGCCATCC AGGTCAGAAA GCTCTCCTAC ATTATAATGA AGAGGCTGTC
 901 CAGATAAATC CCAAGTGCTT TTACACACCT AAGTGCCACC AAGATAGAAA TGATTTGCTC
 961 AACAGTGCTC TGGACATAAA AGAATTCTTC GATCACAAAA ATGGAACCCC CTTTTCATGC
1021 TTCTACAGTC CAGCCAGCCA ATCTGAAGAT GTCATTCTTA TAAAAAAGTA TGACCAAATG
1081 GCTATCTTCC ACTGTTTATT TTGGCCTTCA CTGACTCTGC TAGGTGGTGC CCTGATTGTT
1141 GGCATGGTGA GATTAACACA ACACCTGTCC TTACTGTGTG AAAAATATAG CACTGTAGTC
1201 AGAGATGAGG TAGGTGGAAA AGTACCTTAT ATAGAACAGC ATCAGTTCAA ACTGTGCATT
1261 ATGAGGAGGA GCAAAGGAAG AGCAGAGAAA TCTTAA
```

FIG.5A

```
   1 MDFSPSSELG FHFVAFILLT RHRTAFPASG KKRETDYSDG DPLDVHKRLP SSTGEDRAVM
  61 LGFAMMGFSV LMFFLLGTTI LKPFMLSIQR EESTCTAIHT DIMDDWLDCA FTCGVHCHGQ
 121 GKYPCLQVFV NLSHPGQKAL LHYNEEAVQI NPKCFYTPKC HQDRNDLLNS ALDIKEFFDH
 181 KNGTPFSCFY SPASQSEDVI LIKKYDQMAI FHCLFWPSLT LLGGALIVGM VRLTQHLSLL
 241 CEKYSTVVRD EVGGKVPYIE QHQFKLCIMR RSKGRAEKS
```

FIG.5B

```
Bkb1   ...........................................MVK.KLVM
BKb2   ........................MFIWTSGRTSSSYRHDEKRNIYQKIRDHDLLDKRKTVT
BKb3a  .........MQPFSIPVQITLQGSRRRQGRTAFPASGKKRETDYS...DGDPLDVHKRLP
BKb3b  ...............................MTAFPASGKKRETDYS...DGDPLDVHKRLP
BKb3c  ...........MFPLLYELTAVSPSPFPQRTAFPASGKKRETDYS...DGDPLDVHKRLP
BKb3d  MDFSPSSELGFHFVAFILLTRH.......RTAFPASGKKRETDYS...DGDPLDVHKRLP

Bkb1   AQKRGETRALCLGVTMVVCAVITYYILVTTVLPLYQKSVWTQESKCHLI......ET.NI
BKb2   ALKAGEDRAILLGLAMMVCSIMMYFLLGITLLRSYMQSVWTEESQCTLLNASIT.ETFNC
BKb3a  SS.TGEDRAVMLGFAMMGFSVLMFFLLGTTILKPFMLSIQREESTCTAIHTDIMDDWLDC
BKb3b  SS.TGEDRAVMLGFAMMGFSVLMFFLLGTTILKPFMLSIQREESTCTAIHTDIMDDWLDC
BKb3c  SS.TGEDPAVMLGFAMMGFSVLMFFLLGTTILKPFMLSIQREESTCTAIHTDIMDDWLDC
BKb3d  SS.TGEDPAVMLGFAMMGFSVLMFFLLGTTILKPFMLSIQREESTCTAIHTDIMDDWLDC

Bkb1   RDQEELKGKKVPQYPCL..WVNVS.AAGRWAVLYHTEDTRDQNQQCSYIPGSV..DNYQT
BKb2   SFSCGPDCWKLSQYPCLQVYVNLTSS.GEKLLLYHTEETIKINQKCSYIPKCG..KNFEE
BKb3a  AFTCGVHCHGQGKYPCLQVFVNLS.HPGQKALLHYNEEAVQINPKCFYTPKCHQDRNDLL
BKb3b  AFTCGVHCHGQGKYPCLQVFVNLS.HPGQKALLHYNEEAVQINPKCFYTPKCHQDRNDLL
BKb3c  AFTCGVHCHGQGKYPCLQVFVNLS.HPGQKALLHYNEEAVQINPKCFYTPKCHQDRSDLL
BKb3d  AFTCGVHCHGQGKYPCLQVFVNLS.HPGQKALLHYNEEAVQINPKCFYTPKCHQDRSDLL

Bkb1   ARADVEKVRAKFQEQQVFYCFSAPRGNETSVLFQRLYGPQALLFSLFWPTFLLTGGLLII
BKb2   SMSLVNVVMENFRKYQHFSCYSDPEGNQKSVILTKLYSSNVLFHSLFWPTCMMAGGVAIV
BKb3a  NSALDIKEFFDHKNGTPFSCFYSPASQSEDVILIKKYDQMAIFHCLFWPSLTLLGGALIV
BKb3b  NSALDIKEFFDHKNGTPFSCFYSPASOSEDVILIKKYDQMAIFHCLFWPSLTLLGGALIV
BKb3c  NSALDIKEFFDHKNGTPFSCFYSPASQSEDVILIKKYDQMAIFHCLFWPSLTLLGGALIV
BKb3d  NSALDIKEFFDHKNGTPFSCFYSPASQSEDVILIKKYDQMAIFHCLFWPSTTLLGGALIV

BKb1   AMVKSNQYLSILAAQK..................................
BKb2   AMVKLTQYLSLLCERIQRINR.............................
BKb3a  GMVRLTQHLSLLCEKYSTVVRDEVGGKVPYIEQHQFKLCIMRRSKGRAEKS
BKb3b  GMVRLTQHLSLLCEKYSTVVRDEVGGKVPYIEQHQFKLCIMRRSKGRAEKS
BKb3c  GMVRLTQHLSLLCEKYSTVVRDEVGGKVPYIEQHQFKLCIMRRSKGPAEKS
BKb3d  GMVRLTQHLSLLCEKYSTVVRDEVGGKVPYIEQHQFKLCIMRRSKGRAEKS
```

FIG.6

FIG. 7A-1

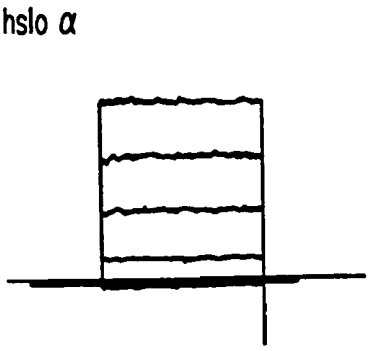


FIG. 7A-2

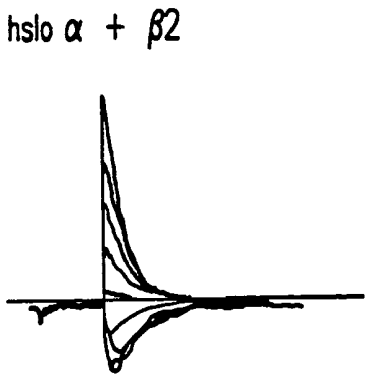


FIG. 7A-3

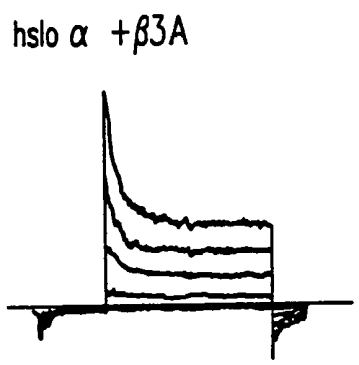


FIG. 7A-4

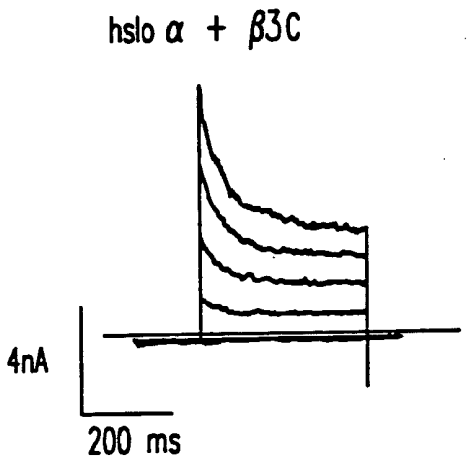


FIG. 7B

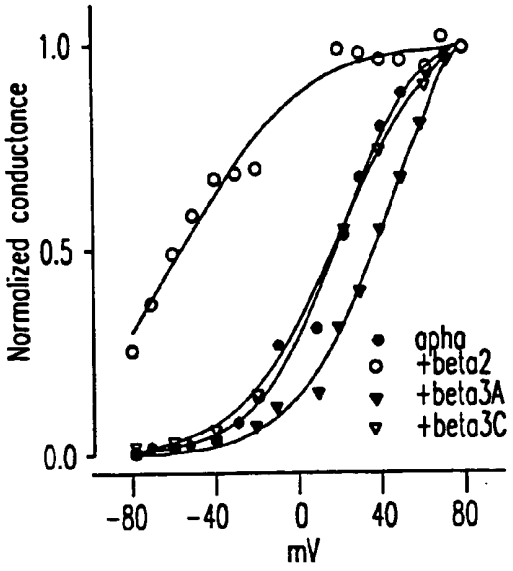


FIG. 7C-1

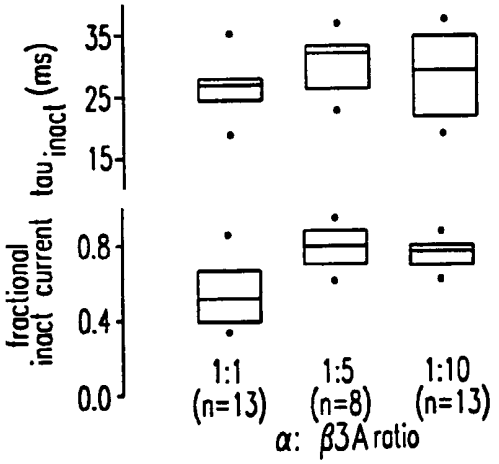
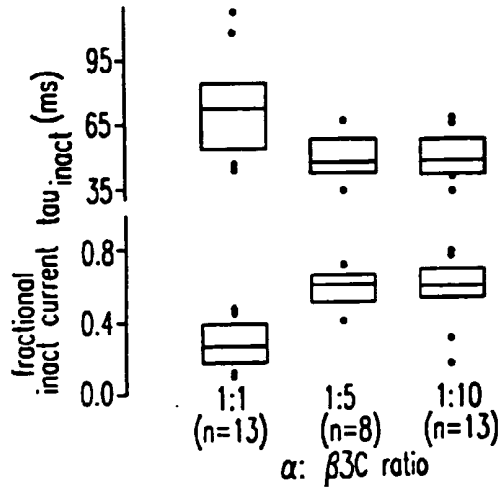


FIG. 7C-2



```
   1 aatcatgtga gttcatcatt ataaaacagc tgatttataa caactgattt taaagtgggt
  61 tgttagcttg aagataaatg agatagggca tgattcaggt ttctgtgtac acactatatt
 121 ctaatgaaag taattacaat tatcttctaa acagctgcag atatttttc tataaacaat
 181 tttagatgtt acattgttaa atgttagctt tcaaattctt cacattttaa ctcaatgaag
 241 tccttttag caaacttaca ggaatcattg tatcattcag ctattaaata aggaattgtt
 301 ctaattcaca tcttaattaa gaactttact aggttatatc ttttgcaagt atgagaataa
 361 tctagataga aggactagaa ttggattaaa ggtgatccag ataaaaatag taattctaat
 421 gaggaaattt tttaacattg aaaatagtac cctgtttatt ttttttaatg ttagagtcaa
 481 accattgcac attactgtgt aaaaatacac aaagatggta acttgctgct aaagcccttt
 541 ttacttttga atctttgtat ttttttcacc tttgtaattt taagttgtgc ttttatcact
 601 catttgtttt ctatctttat ttctgtttgg gttaaagtta caaacagcaa gtttttgtgt
 661 ttaaattcct gaaaatgttg cgatggaatt acagtaatgt gttacggctt gggcccctcg
 721 caggagtgta tctcgcagtc agcagtgaaa acaaagttcg aacagcacac tatcagagct
 781 aaacagatac tagctactgt gaaaaacata atggattcag taaacctggc agctgaagat
 841 aaaaggtatg agttcatttt gttgcaacat aaaaatgtta gttttttttgt cagacttttg
 901 ttaagatgag gttttaaatt gttgtactat aaatactttt tacactgaaa tcaaccttgt
 961 gggtttaact gatgcctact tctatatttt taagtgcgta tttgaaagtg tcgatattta
1021 ttctgcaacc ttgcacacat catttatatc ccctggacct cactattcct gtgtatgaat
1081 tgtgattttt aaaatgtttt gtggctttaa aatatctttg aagcagtgga agaaccattt
1141 cttcaagcag atttttatga agaagtccaa aacaggtgat cctctttctg cattcaaccc
1201 acgacccatt ttttaatatg gtcatacatt ggtattttga aatcactatt ttatactagt
1261 tgtgtttgtt tattgcctgg tgaacaaaca aggcgatgct acctgccaac cagaaagttg
1321 atttggtaag cagtaccctt tatgttatac tgtacggcat tattttatac tatttgttac
1381 cttgtaaagg tggcaatgaa tacatgaaaa taccagcatt atcgatacaa agtataattt
1441 tcacaaactt ggtatgcttg aaacacagat cgggatgtta ttagatgtga caaaagctaa
1501 tgatgttaag cctcgatagg accttcagtg gacttaagcc agttgaagga aacataaacg
1561 catcaaagct taagtccaag atgacataac attggaattt taaatgtatt tgctctttca
1621 gggacaaaga ccccttcatg tccctgtctg ttgctttata atggtacttt cttaggaatg
1681 aattaagttc aaatgcagag aattgccagc atataaaacg caaggatcag aaccctgagt
1741 ttgaactcag cccctgtgta cagcctccgt gtggcctctg tttaattaga tcgtgctgct
1801 atagcagttc cttctagctc agttgctttg atgtagtacc caaattttgg cctaaaagtg
1861 atttaattag taataatttt taaagatata ggatgttgaa caaagtatag cacaaagaag
1921 atgtgatttg aggattgtat aatcataatg tcctgggaac ttcttaagta aaagatcttc
1981 ttaaatggat ctcaggtctt tattttcctg tatcagccag agttgaacaa acttttgttt
2041 aaaaaaagag ccagatagta aatattctag gctttatggg ccataaagtc tcttggagct
2101 actcatttct acagttttag agcaaaagca gccatagata gataataggt aaatgaatgg
2161 gtgtggctgt gtttcaagaa aactttaaca aaagctggca gctggctaga tttggcttgt
2221 aaggtgctat atgctgaccc ctgcttatta actaaagtca gtattgcatt ctgttttgcc
2281 tgttcatatc tatgaagact tagaattctt attcatcctt tctgggattc aggtgccaca
2341 tgggcagaga aacgtggttt ctatcaaatc atctatataa aatactttat aagtgaaata
2401 ttacttgaat cctttgagat gttacaagtt tttttttcc ttgagtcatg ctaatagatg
2461 gttcaataca tgaatgagtc ccttgctgaa atgctttagg acttcagact accctgaacg
2521 ttgattactc tttatactga aataggcatt attcagtgga agagagggaa gaccaaattg
```

FIG.8A

2581 atagactgga ctttattcga aaccagatga acctttaac actggatgtt aagaaaaaaa
2641 tcaaggaggt taccgaggag gtggcaaaca aagtgggtaa cagtagcttc atgattaaaa
2701 taacctggat ggaaattatt tttgataatg ctaaaaatgt tattcctgtt actttaaaag
2761 aatgttttca gatttttcaa tttaattttt ttgaataatt gataatcttg tttaattatt
2821 ttaactccaa aattttcata ttttcaggtt tcatgtgcaa tgacagatga aatttgtcga
2881 ctgtctgttt tggttgatga attttgttca gagtttcatc ctaatccaga tgtattaaaa
2941 atatataaaa gtgtaagtta aagtatagat aaaattattc agagacagtt tcttattatt
3001 ctataccctc atttatttca tggtttggca tttcagtgta tcagtacaaa atgaaactgt
3061 tagatctctt gtgccctctt gtaataaatg taaactgtct tgtataaaaa gtaaatagaa
3121 aatttatact tagaatgaga taaagatcat tttaggacga qgcacagtgg ctcatgcctg
3181 taatctcaac actttgggag gccgaggtgg gaggatcagt tgggccccag agttcgagac
3241 cagcctgggc aacacagtga gaactctaat ctacaaaaaa cagttaggct ggttgcggtg
3301 gctcccgcct ttaatcccag cactttggga ggctgaggcg gacggatcac gaggtcagga
3361 gttcgagacc agcctgacca acatggtgaa accctgtcta ctaaaaatac aaaaaaaaaa
3421 ttagccggtg gcaggcgcct gtaataccag ctactctgga ggctgaggcg ggagaatcgc
3481 ttgaaaccgg aaggcagaag tggcagtgag ccaagatcac accactgcac tccaacctgg
3541 gcaacaagag caaaactcta tctcaaaaaa aataaaaaat agccaagcat ggcgacacgc
3601 ttctatagtc ccagttactc aggagtctga ggcaggagga tcgcgtgagc ctaggaggtc
3661 aaggctacag tgagtcaaga tcaaagcact ccagcctagg caacaaagca agaccctgtc
3721 tcaaaaaaaa aaaaaaagtc aaattaaaaa gaccattttg gcatttactg aatattttat
3781 gtctttataa aaactacata ctttctggag aaaaaataat atggatattt accattgtta
3841 acaggaatta aataagcaca tagaggatgg tatgggaaga aatttggctg atcgatgcac
3901 cgatgaagta aacgccttag tgcttcagac ccagcaagaa attattggta atatttatgt
3961 ctacaaggtc atgtctggtt tgttttttca ttcatgactg gtgaagagct tattttcctt
4021 taggcattcc attgaaggta aaacatttac cattcttatc ttaagtgttg taattttgtt
4081 ctttctagaa aatttgaagc cattacttcc agctggtata caggataaac tacatacact
4141 gatcccttgc aagaaatttg atctcagtta taatctaaat taccacaagt tatgttcaga
4201 ttttcaagag gatattgtat ttccctttt cccttgggct ggtcttccct tgtacatcga
4261 tttttgggcc tagaaatgct caaagggtgc tcctaggatt atcagagcct atctttcagg
4321 tatgtatctt tgaatctacc aattaagact ctcctttatt attttgttta tgtggttttt
4381 ctataataaa actagcttta caaaatctgc acatttaaaa gctagtatct atctcttaga
4441 gccatttctg aggtagatat ttagtttgag catctccaat ctgaaaatcc ttaaaggtcc
4501 aaaatgaaaa acttttgag tacggtgaca ccacaggtgg aaaattccac accagacctc
4561 aatgaggtca tagtaaaaac gcaggcacac aacacacagt ttattcagtg tcctcaaggg
4621 aaaaaagact cagcctcttc tagctgcaat atatcttttc cacgtatgcc caaattcccc
4681 cacacaagca cacctatgaa gggtactaaa atggcacatg tgcaggtcca gtgcaccaac
4741 agcattttcc ccatgatgcc ccacaagggg ccaagacctt tgtgcattac ccagtatggg
4801 cttctttact tcctgtcttt tctctggtgt aaagatactg tttaaaaaaa attttttttt
4861 gagtggggat gcgtttgaga cagagacctg cgctgtggct caggctggag tgcagtggtg
4921 caatcttggc tcactgcaac ctccacctcc cgggttcatg cgattctcct gcctcagcct
4981 cctgagcagc tgggattata cgcacccacc accacaccca gctaattttt gtatttttag
5041 tagacatggg tttcaccgtg ttggccagac tggtctcgaa ctcctgacct caagtgatcc
5101 acctgcctca gcctcccaaa gtgttgggat tacaggcgtg agccacagca cccaggctgt

FIG.8B

5161 ttaaaatgtt taaaaggcct acagataccc ttgtgggtga cattgataag aaaaagaaga
5221 ggcatttatt tttatttata acacataaat ttaagctgtt ggaaaaactg gacaataatg
5281 taagtgagaa acttcttaca gaagaatgat gttggaatga acaccatata tgacctaaaa
5341 gaaagaacaa ctttcctaaa gttgttcttt aggactaaac tgttgaagtt ccatgctaaa
5401 aatgatgaca gaagttaatg aaaaaaacaa aaacactgca gaaagttaaa aacgaagatc
5461 ttgatcatgc attgaaagag tggatccatc agcattgcag tgaacacgtg ccacttaagg
5521 acatgctgat catgaaataa acatctatta aaatgaactg aaaatcgaag ggaactgtga
5581 gtattcaata ggctggttag agaaatttaa ggtaagatag cattaacttt tctaaaggtt
5641 tgtggtgtta aagcatcttg atcacaaaac agcagagaaa ttcattgatg aatttgccaa
5701 gattgtctct gatgaaaatc tgactccaga acaagtctat aatgctgatg aaacatcact
5761 gttttgccat tattgaccca gaaagacact gactacagct gaggagacag cccctacaag
5821 aataaaggat gcaaagaaca gaataactgt gctgggatgt gctaatgcag caggcataaa
5881 tgtgaactta ctgtgatagg caaaagcttg catcctcact gttttcaagg aatgcatttt
5941 ttttactagt ccattattgt actaacaaaa aggcatggat ccctagggac atcttttctg
6001 attggtttca caaacatttt ataccagctt gtgtgcactt gcagggaagc taggccggat
6061 gatgactgca agattttgtt attccttgac aactattctg ctcatcgtct agctgaaatt
6121 cttattaaaa aaagtgtttc attatttcaa ccttgtgacc agggtattct acgatcaatg
6181 aagagtaaat acaaaaacct tttttgggca gcatgctagc agcagtgaac tgaggcctgg
6241 gtgtggaagg ttttcagaag gagtttagaa tgaaagatgc catagatgct tttgccagca
6301 caaggagttt aggatgaagg atgccatata tgccagcact tggaagacag tgactcagtt
6361 gtgtatgcct gcagcctctg cctgtcacta catttattga tgatgaggag caaattggtg
6421 actttgaagt atgtcaagtg agctccttat gcaaaaaata atatcttcag agtccatccg
6481 taagctggaa gaaggatata tcaaagaagt gtttgacatt gataatgagg tttcagttgt
6541 tcattcatta actgatggca aaatagctaa aatgcttcta aatcaaggtg attatgataa
6601 tggtgataat gaagatgatt tcattgacct cgcagaacaa ctgcctatag gcagcatggt
6661 gatgggctta ttgaagcact agagcagcat gtattcataa cagaacaaga aataggttta
6721 taaaatcaag gagagacttc aacaaaaacc attgttaaag aggcaagtag gtggtactgc
6781 aggaaacatt ttaaaaggcc attcagcaca atgtcttatt atgccgagag gacccacttc
6841 ttggtccctc aactgcttct gatgtttctt ctcacctaac aaagtactgt gtaccggaac
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6961 acagctgata caggtgttct ggtgatgcca ctgtgctgct tagtttgaat acgttatttt
7021 tcactgttat taatggtgtg tcttatattt ttactattaa gttcctttgt gtgaatccgt
7081 gtaagaaaat gattgcttgt cagtagtatg taaattcaat caagaatgat ggtgatgcca
7141 aacaaccata gagtgttcac atgggtggct gacatagcaa cacctgtgtt ttctgataag
7201 tcagtgtaca caacctttgt ttcatgcaca aaattattta aatattggat aaaattacct
7261 tcaggctatg catataaggt atatgaagca taaatgaatt ttgtgtttgg acttgggtcc
7321 catcctcaag atagctcatt atatctatga actattcaaa aatcctaaaa aatctgaaat
7381 ctgaaacact tctggtccca agcattttgg ataagggaca ctcaacctgt agtatgctta
7441 agggaaagct tatcctaaag ctttcgtcca atactgttgc aggtggtgct acattcattt
7501 ttgaaacatt tctgtttttct taaaagattt ggttttcaca tttcaaataa actagcataa
7561 atacaagcat ttttttaatt tttttttatt atacttttaa gttctagggt acatgtacac
7621 aacgtgcagg tttgttacat atgtatacat gtgccatgtt agtgtgctgc acccattaac
7681 tcatcattta cattaggtat atctcctaat gctatccctc ctccttcctc ccatcccaca

FIG.8C

7741 acaggccccg gggtgtgatg ttccccttcc tgtgtccaag tgttctcatt gttcaattcc
7801 cacctatgag tgagaacatg cagtgtttgg ttttttgtcc ttgcaatagt ttgctgagaa
7861 tgatggtttc cagcttcatc gatgtcccta caaaggacat gaactcatca ttttttatgg
7921 ctgcatagta ttccatggtg tatatgtgcc acatttttctt aatccagtct atcattgttg
7981 gacatttggg ttggttccaa gtctttgcta ttatgagtag tgctgcagta aacatatgtg
8041 tgcatgtgtc tttatagcac catgatttat attcctttgg gtatataccc agtaatggga
8101 tggctgggtc agatagtatt tctagttcta gatccctgag gaatcaccac actgtcttcc
8161 acaatggttg aactagttta cagtcccacc aacagtgtaa aagtgttcct atttctccac
8221 atcctctcca gcacctgttg tttcctgact ttgtaatgat tgccattcta actggtgtga
8281 gatgatatct cattgtggtt ttgatttgca tttctctgat ggccagtgat gatgaacatt
8341 ttttcacgtg tctattggct gcataaatgt cttcttttga gaagtgtctg ttcatatcct
8401 tcgcccactt tttgatgggg ttgtttttttt cttgtaaatt tgtttgagtt ctctgtagat
8461 tctcgatatt acccctttgt cagatgagta gattgcaaaa attttctccc attctgtagg
8521 ttgcctgttc actctgatgg tagtttcttt tgctgtgcag aagctcttta gtttaatgag
8581 atcccatttg tcaattttgg cttttgttgc cattgccttt ggtgttttag acatgaagtc
8641 cttgcccatg cctgtgtcct gaatgatatt gcctaggttt tcttctaggg tttttatggt
8701 tttaggtcta acatttaagt ctttaatgca tcttgaatta atttttgtat aaggtgtaag
8761 gaagggatcc agtttcagct ttgtacatat agctagccag ttttcccagc accatttgtt
8821 aaatagggaa tcctttcccc atttcttgtt tttgtcaggt ttgtcaaaga tcagatagtt
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9001 ggtagcgtga tgcctccagc tttgttcttt tggcttagga ttgacttggc aatgcgggct
9061 ctttttttggt tccatatgaa ctttaaagta gtttttttcc aattctgtga agaaagtcat
9121 tggtagcttg atggggatgg cattgaatct ataaattacc ttgggcagta tggccatttt
9181 catgatattg attcctccta tccatgagca tggaatgttc ttccatttca cccattcaca
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9301 ttcaaggaga actacaaacc actgctcatc gaaataaaag agaatacaag cattttaaaa
9361 gaaaaacttt aaaaaattta tcaattcagt aatttttttag gaaatttgtg aaattgtaaa
9421 accatcacca caatttagtt ttagtttttg tcacctcaaa gctgttttac tcatttgtag
9481 tcaattatga tttccacctc cagcctcacc actaatctgc cttctgttta tggatttgcc
9541 ctttctggag gtttcctata aatgcaatca tatagaatgt ggccttatat gactggtttc
9601 tttcacttag tataatgttt tcaaggttta tcatcatggt atcagtgttg taggatgtat
9661 cagtacttca tttcttatta cggctgagga atattccatt atagatacaa cactatccat
9721 tcaccagttg atggacatta gggtagtttg tagtttttgg ccattatgaa tactgctata
9781 aatattcatg tacatgtttt ttatgtggac gtgcgttttc atttctcttg agtatatacg
9841 taggttctaa tgggtataga gtagtattcg ttgtaggttt gatttgcatt tccctaatga
9901 ccaatgattt taaacatctt ttttgtgtgc tcactagcca tttgtgtatc ttcttttgtg
9961 aaatgtctat tcaaagcttt tacctatttt taaattattt gggtcctata gagttgcagg
10021 atttctttgc atattctgga tcaaatcctt tctcacatat atgatttcca aatatgtttc
10081 tttatttttt gagacaagga cttgctatgc ccaagctgga gtatggtggt gtgatcacag
10141 ttcactgcca cctctgcttc caaggagctg ggaccacagg catgtgccac cacacctagc
10201 taatttaaaa gaaatttttg tagacacaag gtctcactgt gctgtgctgg ctggtttcaa
10261 actcctggtc tcaagctatc ctcctgccat ggcctcccag agtgctggga ctacaggcat

FIG.8D

10321 aagtcactgc acccagcccc aaatatattt ctcttgcctg tggctgtgtg ttttgaagtt
10381 taagaggttt ttattttgaa gtccttttt ttttttttt ttttttgag actgagtttc
10441 actctgttgt ccagactgga gtgcagtggt gcaatctcgg ctcactgcaa gctctgcctc
10501 ctgggttcac gccattctcc tgcctcagcc tcccaagtag ttgggactac aggcatccac
10561 cacctcgctg ggctaatttt ttttttttat gtatttttat tagagacagg gtttcactgt
10621 gttagccagg atggtctcga tctcctgacc tcgagatcca tccacctcgg cctcccaaag
10681 tgctgggatt acaggcgtga gccaccgcgc ccggccgaag tccttttttt ttttttagt
10741 ttatcatttt ttgttgttgt ttgtttcaag gattgtgctt ttgttgttag ttttttttaaa
10801 aacacacttt gttcctcttg agagttgagg gcctactgtt acatttagac cccattgtat
10861 attatgaaaa tttattgttc atgatatcat tggtccatat tgtagataag aatttccaaa
10921 ttatttttat tttctacata tatttgtggt gtactcatca tttagggtaa tttttccctc
10981 atagatgtgc tactataatt ttagagtttt ataaagataa ctttatagtt gtttaagtgt
11041 aatctttttt cctttctctt ttttttggc agctccctag atctttagct tctactccca
11101 ctgctcctac cactccagca acgccagata atgcatcaca ggaagaactc atgattacat
11161 tagtaacagg attggcgtcc gttacatcta gaacttctat gggcatcatt attgttggag
11221 gagtggtaag aaacattact tttagtataa ttaaaatcga aatgtttgca aggctgcctg
11281 ttagctcaca aaagaaaagg tatacagtat ttacttctat ttataggatc tgtaaaatag
11341 tatgaaaatt tgcaggttaa aagtagtgaa ttaaatatca caagtttcat cttaaatttt
11401 taaaaaacat acatctttag gaatgaacta tcaccaccta gctggtcttt aatacttcct
11461 aagcattttc acatcacagc acacaaagaa atatttgtac gttgaggtaa taagaaaacc
11521 ccaggtgccc tatctagcct tctctgaata tcaaggggat ctgtcttaag tattaaatgt
11581 tatttttagt agtagttctt atttgtttct ttaaaaaatg tatatcatta acagcatgga
11641 tatgcttgtt ttggtttttg aaaggtctga cctacaatat ttaacattgt tttatttttg
11701 agatacattg aatcaggata agtctggcag cttataaagc tctccctcat ttgtgtgctg
11761 atccctttta agcctgattg atttctggta acttcaggtt ttctcatgga gaaggaatat
11821 acatttttag aaaatgtatc taactcaaag atccataggg aactaaaatg cttttaaatg
11881 tactctccaa agtggtgttt ttccttctca gactaagcta tgactttatc ttacagattt
11941 ggaaaactat aggctggaaa ctcctatctg tttcattaac tatgtatgga gctttgtatc
12001 tttatgaaag actgagctgg accaccccat gccaagggag cgagccttta aacagcagtt
12061 tgtaaactat gcaactgaaa aactgaggat gattgttagc tccacgagtg caaactgcag
12121 tcaccaagta aaacagtaag ttggaaggtg catctttcct ttaaaaaaaa gttactgaaa
12181 tatgacatac atgcagaaaa agcacaaaat aagtgtattg ctcaaagaat tatcacaaaa
12241 tgaacatgtt tcgtgatggc cataaatgag aaaaaataga acaatactaa acctcactgc
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12361 acattagaaa attggtagat aggaactctc aagaactctg gaggggttta aaaagatagt
12421 tcttaatttt ttttcttttt tttcagagat agggtctctg tcgcccaggc tggagggcag
12481 tggcacaatc tggctcactg cagcctcgaa tcttgggctc aagtgatctt actgcctcag
12541 cctcccacgt agctgggacc acaggtgtgt gccaccacac caggataatt ttttaatttt
12601 tttttttttct ttgagacagg gactcaatat gttgcccggg ttggtcttga ccacctgggc
12661 tcaagtaatc ctccctcctc aagcctcctg agtagctgag attataggca tgagccacca
12721 tgcccaactc aaaagatctt cagcagacct attctaaatt tatgtacctg gctgggcaag
12781 gtggctcacg cctataatcc cagcacattg ggaggctgag gcaggcggat cacttgaggt
12841 cgggagttcg agaacagcct ggccaacatg gtgaaacccc atctctacta aaaacacaaa

FIG.8E

12901 aattagccgg gcatggtagc acatgcctgt aatctcagct agttgggagg ctgaggcaca
12961 agaatcgctt gaccctggaa ggcagaggtt gtagtgagcc gagatcacat cactgaactc
13021 cagcctgggc gacagagtga gactctgtca cacatacaaa aaaaattaag cactggatat
13081 agatttattt ttctattctt tgtctttttc tccttagaaa gtgaaacaga aaaaaaacaa
13141 aataaaataa cttctaattg attaagaatt caggttattt gtgttcttat taataggggt
13201 tattctataa catttaggaa tgcatcaaaa ttcatgatca gatatcactt gccaagaatg
13261 ggggcttcat cagatccgga atagaattta tctaaaagtg atcaagacat gcagacttat
13321 aaaaagctat gaacatcctg tctgtataac aacttggcca gcaacattcc tggcgcaaag
13381 ggctaaggct ccttcaagcc ttgagaataa gacacttaaa agaataagcc caagctcctc
13441 ctgagcgagg aggccgaata ttgtcagtag aagcatggac ttttggatgt gatctgttct
13501 ggagccccgg acctagccct tgttactttg tgatttttgc acaagttcct cggactctct
13561 ggtcttctgt gtcctcatct gctgaatgcg caaaaagttc ctacctcctc aagttctgtg
13621 tctgaaggac attatgttct catagcactg agcacaatcc ctggcacatg gttactcagg
13681 gcacccaagt tatcattatg tgtctaggga aagttgggtt gggcatgcag ttgttgaatt
13741 ctcttctttc tgggtgagcg ctgcctctca gcagctgatg ggggaatcct tgcattattg
13801 tcactccagg agagaagata cctgcttcct gcaagcaaac ttacggtttc atacacttta
13861 ttggatctca aaggcagatc tttttttgtt ttgttttgct ttcttgagat ggagtttcgc
13921 tctcgttgcc caggctggag tgcaatggca cgatctgatc gtggctcact gcaatctctg
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14161 ggattacggt ggtgagccac cacacccggc ttaaaggcag atcttaaaag cacattaaat
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14281 acctgagact agaaatggtg cttaggttcc taagcccagg cctccaaggt ctatttactg
14341 tataatgtga gctgatgtct cccaaagtat aaatgtaggg tcacctgtgt taggatcata
14401 tgaagggctt tttaaaatgc aagttcttgg gtcctttacc ctaattgttt tatcagaatc
14461 tctagagatg ggacctggga atctgtattt aacagaggat cacacctgag ttcaagaacc
14521 actcatgtag tagaacaatt acctcaactt aaaatatgaa atgtatctgt agcaagtgcc
14581 acctggtaaa gacttgatca cagtggattt caaacaagac aaagtattga gggctgttga
14641 actgtcaaag aatttcagct attatttcta ttagtttctg cctcactatc catcgagttg
14701 tttgtatgcc aggataggcc aagttctctg gctcttgtga gcttgtgtaa gtcagtgtag
14761 ctctctgctc ttgaaaaagc ccaaaaagtg aattaacatt tgtatagact tagaattgta
14821 actagctcat aaatagtagc cactagtatt atcactcaga gcaggaaaag catctgcaca
14881 gaggtgacgc tggtttctct gatgtgagcc tagttcaggc agtcaggtgc ccatttgatt
14941 agcaagatgg ctggagataa taatcagggt aaaggaaagg aaagatccca tcgaaggctc
15001 cagaagtttg tggcacagat catacttcct tgttgtttgt tttcctgcaa agagaatgta
15061 agatcagatg tgactttact tttgacagtt tgaattcttg ctcacatcag caaaaattct
15121 ccatattgga ataactgtcc agttaggggt ttacttattc tcctacgaac aaatatagat
15181 agtcacgcaa agaacatagg ctgttgtgta aattttaggt tgttgtcaat gatttgtgca
15241 tctctaaatt ggaaaacaca gacatagttt tcatgaacat ggagaatttc agctacaaaa
15301 taattcttag ccataatagg tattgtatat ttaattgaga gaatgtgaaa aacaatgagg
15361 aagtagtttc tccagtatgg tgaaaggcaa agagggttct ttttttccc ctaagtaaag
15421 catctactaa atgcaaaaga aatgattgtg gactctggaa tctggaatcc acgatgctag

FIG.8F

15481 cactttgcag taatagcctc tttcatatat agatctcaca acagtttgta gacactaagt
15541 ttttccatgc tcctgcaact cacactgagt ttttatttac tccctttta ctctggaaaa
15601 gcagggcagg aagtttttaa agggttcctt gcagttacaa agctagaatt tgaacccagt
15661 gtcaaagcct ctgggtcaga ttgggagggg gcccacgttg tgaacttgac agttaattca
15721 tggttgctgt tttatggagc aggaagtgac ctttagtgac tcagaggaag gaataagctg
15781 agggagggtc caggagacca gaggtacagt gcctggcatc tcttatcact gcattctaag
15841 tggcttagcc ttgctgtgtt cctgaatgca cacattggga ttgcaactca caccgaccac
15901 tttaggttct tcttgagaga taggctgtga gtcctgaatg gagtgtgtgt gtgtaggggg
15961 cttggctttc aggccaagag tggagtgagt gtggtacttg gtggacagag aggccagttg
16021 gttatcaagt gaaggggcgt tagggaaaaa gttcagaggg caaaaagcta tgttgctcct
16081 tctcacttat tgtccatgag tattttcttt taaatggaac cggaatcata agataagaat
16141 gaccaggagg ctctggtaaa ggcaggtgca agttttgtgt gacaagatcc tcatggacta
16201 aaaaatgatc atatttctga ggttgaatca aatacataaa agcagcagca aatcacacac
16261 ttcttcgaag agagttacca agttgcaggg gaaatttatt agcacaaata agtctgaaaa
16321 ggaaaagcta tccatatata caaggttaag ttggaagaag gaggcagaaa gaagaaattc
16381 cagttgttat cattattttt tattccaaat tgcatctgtt cactaaaata cttcggactg
16441 ggtgcagtgg cttacaccta taatcccagc actttgggag actaggcggg cagatcactt
16501 gaggtcaggc attcgagacc agcctggtca acatgatgaa accctgtctt tactaaaaat
16561 acaaaaatta gccagtcgtg gtggcacatg cctgtaatcc cagctactcg ggaggctgag
16621 gcaggagaat cgcttgaacc tgggaggtgg aggttgcagt gagccaagat tgagccagtg
16681 cactccggcc tgggtgacag agggagactc catctcaaaa aaacccaaac aaatatttta
16741 taaaatagaa atagaaaaat aatacaaatg aaaagtctat taagtatata tttttattaa
16801 gcaatattaa aatgactgca agtaagagtt tatatagcta tatgtacatg gatatttata
16861 gatgagatta cgcttacatt cttgcccaca ccatcttgga aaatgttaag ataatatcgc
16921 cttgactgaa aaacatacag caaacatggt ctctttggca ttctgtcatc cacatctaca
16981 ggtgcctgta gcaatgtgtg gtactataat ataatggtaa ttgatgttcc taatttggga
17041 gtgtggaaag atccccaaat gtcttttaag tcatcagaga aagataaaat aatatttgat
17101 acagcttttc ttaaaatttg agataatttt aatggcgagt tattttatgg ccctttgtat
17161 cttgaaaaat tgggaaatca catatggttt aaaagcgaat tatcttaatt ggaacatgcc
17221 attaactaga aaacccatta tttcagcttg cactctadca gacaatacgt ggaaaaggaa
17281 acgcggccag ggcaaaacat ttcctcttct tataaacctt gaactgagta cgtccctcac
17341 caattataga gggtcccctt gggcctcaga actttccaca agcgttgagg tctctatggc
17401 gatgctcccg gctgccgagg cggaaacaca ggtgatgagg tggcggcaag cacagtgcaa
17461 agagagagaa gcagcttcgg ctgcagcaaa ccacgcaggt ccttcttgat catctagaac
17521 tgaccgctcc gccttgccag gagtctgcag aaccacgtgg ctagcctgcc tgaagttctc
17581 acctctccag gaaggcgggg ggcttctaat ggctgcagct gcgctggggg ctgggggctc
17641 ccgctgggac tccacttccg tggatgtcta agcttcacct ttcttgcgcc cgcaggggca
17701 tgactcaggt gaaagggagc cattttctca gacccctggc ctcatgcagc ccttcagcat
17761 ccccgtgcaa atcacacttc agggcagccg gaggcgccag gggaggtaag tcacttccgg
17821 aagctctgcc ggtagtggga atctggctga acaagcagtt gcaagaagag gggacatctc
17881 gagcttgggg agtgagtgtt tccttttttct ctgaggatgc ccacttgcca tgcctcccag
17941 ggtacccagc aggcttcccc agtagcactc acatcacggg gctgcagcct ttcctgttgg
18001 ctctatcctc taggttgcca gttcttggag actggagacc ttttaaactc acctgtagct

FIG.8G

18061 ccccagcact gatcatagcc cagcccatag ttggtgctca agaatgatct gttggttgaa
18121 tgaggaatga agaaattaga ccagcatttg gtcccattgg tgaagccctg gagtcacagc
18181 ccttggattc aaacccagct caccacttaa tcagccatat gactgggcag gtcccattgg
18241 tgaagccctg gagtcacagc ccttggattc aaacccagct caccacttaa tcagccatat
18301 gactgggcaa gtcacttaac ttctctgttt gcctcatctc cttatctgtg aaatgcagat
18361 agtaagagcc cctgcgtgtc tcagcacagt ataccacatc ctctctaaac tatactgtta
18421 taccgggtat acgctctaca cctccctaaa ctctagcctt ttagctattg ttattaccca
18481 ccctactctt ctccttaaag ggggaagtga gagattattt tcaggacccg ttttctcccc
18541 agggaaatga aaagcaaaga agctgaaagc ctctagtgac ctgcacccct tttgcctgcc
18601 ctagggttgg ggcatggggc agtcaatgtg ggaaccaaaa tggagagaag agtgatgcct
18661 gagggtgttg gacaaatggg atactaaaac cttttgtgcc aggcgcggtg gctcacacct
18721 gtaatcccag cactttggga ggccgaggtg agtggatcac ctgaggtcag gagttcgaga
18781 gcagcctggc caacatggtg aaaccccgtc tctactaaaa atacaaaaat tagctgggca
18841 tggtggtggc gcgcctgtaa tcccagctac tcaggaggct gaggctggag aattgcttga
18901 acctgggagg aggaggttgc agtgagcaga gattgtgcca ttgcactcca gcctgggcga
18961 caagagccaa actctgtctc aaacaaagaa aaacctgttg ggaggggtac atttcagaac
19021 caggttcact cactatctgg aaatatgcat gatttattat tggctctagt ggagttggga
19081 gctgagaatt ggaaaacatt aaagatggta atggtcatca tgttactcac ttccattatc
19141 acttaactgc actcaggggt atttgagagg gagcatgagg agagtgagat acaggagttt
19201 ggataagatg ggggttcagg gaagaaggac cagacagact acagaggaaa gaaaggtgtt
19261 cttctcgcta gacatgaacc aatttttttg tgaacagaca attaaaatga attactttat
19321 ggcaaaagat caaatgacaa acatgcaagc aaacaagttt tagtgtccca tacgtcacac
19381 aattaactag atataaaggc agttgtgttg tcatcaaagc aaactactgt atcccatttt
19441 catttctgaa atgcacaact gaattattgc tatttcctct tgctgaactt gatgaactat
19501 gttgacttaa ccttatttgc tgtttcaaaa taagttgtta aataatgttg taattaaaaa
19561 atagaagagt aaaaataatt accaagggtc tcctctgtaa ccaaaacgaa ttacagggaa
19621 atattaatat agtgtacttc atgtttagac attcatttca catacacccg tgaaatgaat
19681 gtcagatgaa ttatgaagtt ttgatgagcc acagcatgtt tctaaggaat acttcttgca
19741 aaagtttcag tgctggaggg agaggctgct ggcttctgtg gggatcacac ccaggtgagt
19801 gtgttcaggc tgtttgtaat tgagtttgcc tcaggctaag ccagaagctg cctgtagcca
19861 tgtgtcgtac ttgggctggg tgggaaagtc agtcctatct gcagtgaaga gaatagaagg
19921 tggtggatat tgccttgatt atgaataaaa cagctcaagg taatacactg gttagaagcg
19981 gacatgtatt actggcagaa aaaggaatca atatgctttt tatccatctt cctgactaga
20041 aagcaaacta gatcatactt aagtgctttg aggtccttgg atgaaagatg cttgtaaata
20101 caacaaagtt aattacaagg ctgtttatgg tctgagaaaa ctggaaacaa cctaataata
20161 ttcaataata aggaaatggt taaggaaata tggcatatct aattgatgga acattatgta
20221 gccaatagga ttacaaagaa ttgttaatga catgggaaag tgcttattat gttaagtgaa
20281 aaagataaga ttaacaaaaa aatctcaaat catatctaat gtgatctcat tctgtttaaa
20341 acaatatagg ccaggtgcgg tggattatgc ctataatctc agcactttgg gaggccgagg
20401 caggtggatc acctgaggtc aagatttcga gactagcctg gccaacatgg tgaaaacccg
20461 tttctactaa aaacaaaaaa attagctggg cgtggtagcc ggcgcctata atcccagcta
20521 cttgggaggc tgaggcagga gaattgcttg aacccgggaa gcagaggttg tagtgagctg
20581 agatcatgcc actgcactgc agcctggatg acagagtgag actccatctc aaaaaaaaaa

FIG.8H

20641 aaaaaaaaaa aaagaaaaga aaaagaaaaa caaaacagaa acaaaaaaca aaaaacaaaa
20701 aacctaatat agagcaggag gggattaacc cagcaaadca agtgcacaat cattaccttt
20761 aagtgttgtg ggtgagtttt tgttctcttc tgtacatttt tttttgtatt tttcaagttt
20821 catacaatga gcatataaaa atatattact ttcatgaatc atttgacatt tgttgaggaa
20881 ttctttgtgg ctaagtttgt agtcaggtct tgagaggtga caacgtgctg gcagcccttg
20941 cagccctcgc tcgctctccg cgcctcctcg gccttggcgc ccactctggc cgcgcctgag
21001 gagcccctttt ctgggctggc caaggctaga gccggctccc tcagcttgca gggaggtggg
21061 gagggagagg cgcgggcggg aacccgggct gtgcgcggag cttgcgggcc agtccgagtt
21121 ccaggtgggc gtggactcag cgggccagca ctccgagtgg ccgaccggcc tacaagccac
21181 ggacagtgag gggcttagca cctgggccag cagctgctgt gctcaatttc tcacagggcc
21241 ttaggtgcct ccccgcgggg cagggcttgg gacctgcagc ccgccatacc tgagcctccc
21301 cccgcctccg tgggctcctg tgccgcccga gcctccctga tgagcgctgc cccctgctcc
21361 acggcaccca gtcccatcca ccactcaagg tctgaggagt gcgggcacac gcacaggact
21421 ggcaggcagc tccacctgtg gccccggtgc gggatccact gggtgaagcc agctgggctc
21481 ctgagtctgg tggggacttg gagaacgttt atgtttagct aagagattgt aaatacacca
21541 attggtactg tgtatctagc tcaaggttta taaacacacc aatcagcacc ctgtatctag
21601 ctcagggttt gtgaatgcac caatcgacac tgtatctagc tactctggtg gggacttgga
21661 aaacgtttgt gtccacactc tgtatctagc taatctagtg gggatgtgga gaacctttgt
21721 gtctagctca gggattgtaa acgcaccaat cagcaccctg tcaaaatggg ccaattagct
21781 ctctgtaaaa tggaccaatc ggctctctgt aaaatggacc aatcagcagg atgtgggtgg
21841 ggccagataa gagaataaaa gtaggctgcc ccagccagca gtggcaacct gctcaggtcc
21901 ctttccacgc tgtggaggat ttgttccttt gctctttgca ataaatcttg gtactgctcg
21961 ttctttgggt ccacgctgcc tttatgagct gtaacactca ctgcgaaggt ctgcagcttc
22021 actcctgaag ccagggagac cacgaaccca tcgggaggaa tgaacaactc cagaggcgcc
22081 gccttaagag ctataacact cactgcgaag gtccgcggct tcattcttga agtcggtgag
22141 accaagaacc caccaattcc ggacacagtt tcataaatgt tccatacatg cttgagaata
22201 atatatattc tgtagaagtg agtattctat atttatcatt tagataaaac ttgttaattg
22261 ctttacttaa atctattacc ctactggttt gttcaggtca agctatctaa attactgggg
22321 gagtgtataa aaatacatca taatctgata gtggattttg tctatttctt cttgtagttt
22381 aatcagtgaa acaatgctat caggtaccta caaattagca ttgttacatt ttccgtgtga
22441 attgagcctt taatcagtgt taaaacactt atttttaaat ttctaataaa gctttttaat
22501 ttttaacatt catgtttgct ttttgttaca ttttgcctat aaatttcacc ctttttgtat
22561 ctgtgtttta gatgtatctt ttgtaaaaac atgcatatag atagttctcc atttacagtg
22621 ggattacatc ctgacaaatc catcataagt ggaaaatact gtaagttgaa aacttcagag
22681 tattagctat ttaccctcat gattgtgtgg cagactggga actatggctg gctgccactg
22741 cccagcatct caagagagta gggtactgca tatcgctagc ccaggaaata atgcaaattc
22801 aaaatttgag gtacagtttc tactgaattc atattgcttt cacaccatag taatgtaaaa
22861 aaattgcaag tgtggggcgg ggcgggccat ggggcgggcg gaggcgcccg agccccgctt
22921 cccccgcgcc attccacccc cggccaggct cagcccgcgg ccacctacgc cccgcccctg
22981 ccggctgcgc ccgagcccag tcccgcgagc cgctccccgg cgggctggct cttggccccg
23041 gaagcgcgag cgttcacttc gcggcgagtg gctccgtctc cgcggacaga gcgcgcgccc
23101 cctggcccgg cccgcggggg ggggctccgg cacggtcccc gagcgtttcc cgccgggtgg
23161 agcgggccga gcccagcagg ttgaccagcc ccgcggccac gcagagccgg gagatctact

FIG.8I

23221 gtttgagcgc ggaagcgcag aggctggcgg aggcccggct cgccgcaaaa cgggaggccc
23281 gcgcggaggc tcgtgagatc cccatgaagg agctggagcg gcagcagaag gaggtagaag
23341 agagaccaga aaaatatttt actgagaagg ggtctcgtaa catgctgggc ccgtctgcag
23401 ccacgctggc ctttctgggt gggacttcct ctcagagagg cagcggagac acctctatat
23461 ccatcgacac cgaggcgtcc atcagggaaa tcaaggactc tctagcagaa gttgaagaga
23521 aatgtaagaa ggctatgttt tccaatgctc agttagacaa tgaaaacaca aacttcattt
23581 accaagttga caccctgaaa gatatgttgc tggagattga agaacagctg gctgaatata
23641 ggcggcagta cgaagagaaa aacaaataat ttgaaaggga aaaacacgcc cacagtatac
23701 tgcagtttca gtttgctgaa gtcaaggagg ccctgaagca aacagaggaa atgctcgaga
23761 aacatggaat aatcctaaat tcagaaatag ttaccaatgg agagacttcc gacactctca
23821 gtaatgtttg ataccaagat cctaccaaga tgacgaaaga agagttaaat gccctcaagt
23881 cgacagggga tgggacccta ggaaagccag tgaggtggag gtgaagaatg aaatcgtggc
23941 gaatgtgggg aaaagagaaa tcttgcacaa tactgagaaa gaacaacaca cagaggacac
24001 agtgaaggat tgtgtggaca tagaggtatt cactgctggt gagaataccg aggaccagaa
24061 atcctctgaa gacactgccc cattcctagg aaccttagca ggtgctacct atgaggaaca
24121 ggttcaaagc caaattcttg agagcgcttc tctccctgaa aacacagcac aggttgagtc
24181 aaatgaggtc atgggtgcac cagatgacag gaccagaact ccccttgagc catccaactg
24241 ttggagtgac ttagatggtg ggagccacac agagaatgtg ggagaggcag cggtgactca
24301 ggttggagag caggcagaca cagtggcctc atgtccttta gggcatagtg atgacacagt
24361 ttatcatgat gacagatgta tggtagaggt cccccaacag ttagagacaa gcatagggca
24421 tagtttagag aaagaattca ccaaccagga agcagctgag cccaaggagg ttccagtgca
24481 gagtacagaa gcaggtaggg atcacaacga agaagagggt gaagaaaaag gattaaggga
24541 tgagaaacca atcaagacag aagttcctgg ttctccagca ggaactgaga gcaagggtca
24601 ggaggcgaca ggtccaagta cagtagacac tcaaagtgaa ccctcagata tgaaagagcc
24661 agatgaagaa aagaatgacc aacagggaga ggcattggac tcattgcaga agagaaagaa
24721 caagaaaaag aaaaagaaga ggaaaaaatc cccagtaccc atagaaaccc ttaaagatgt
24781 ttaaaaagag ttaacttatc agaacacaga tttaagtgaa attaaggaag aagagcaggt
24841 aaagtctact gacagaaagt cagcagtgga agcccaaaac gaggtgactg aaaatccaaa
24901 acagaaaatt gcagcagaaa gcagtgaaaa tgttgattgt ccagagaatc ctaaaatgaa
24961 gttggatgga aaacttgacc aagaaggcaa tgatgtaaaa acagcagctg aggaggtact
25021 agctggtaga gacacattag attttgagga tgtcacagtt caatcatcag gcccgagggc
25081 tggtggtgaa gaattagatg aaggtgttgc aaaagataat gctaaaatag ctggtgccac
25141 ttaaagcaat cctgaagaac cagagagcga agatgcagat cactgcaccg tacccaaaaa
25201 tgaaagtccc tcacaggaca ttagtgatgc ctgtgaagca gaaagtacag agaggtgtgg
25261 gatgtcagaa catccaagtc agaccatcag gaaagcttta gacagcaata gcctaaaaaa
25321 ccatgacttg ttggcaccag gaggagagcc gggggacttc aatccagaaa gcagagaaga
25381 taccagagga gggaacgaga agggcaaaag caaagaagac cgtaccatgt cctaagctga
25441 ggcaggcggc aggcgtggtg cacaggaagt ctgagtgtga ggggctcttt tctctccact
25501 gccaatgtaa gtagaatgtt ctaaattcat agagatgcac tgtatgccaa tcaccaggtg
25561 atctactgct ttaagttata gactgttact tgtagatttc catgtaatca ttgagqttat
25621 cacccagatt agaaagacat atttgttatc agtgtacatt ctaattgaga gcatatatcc
25681 agtagtatca aacaataatg tctactgttt atagtccact taataaaaat agaagcattt
25741 accatttgcc ttaggctgat aggaatgtga atattcttga ccaaatatat cagcatctaa

FIG.8J

25801 ttgaaatgac caaatagcat tcttagactt ctgtattatg aatataattg atatttaaat
25861 taatgtcttg ttcatatatg tgtactttca tatttgattt taaaatatac attataacct
25921 gtatggtatt ttatttaaag gagataaacc gccaaatagc aaataggtca ctgaaaagat
25981 ttgcacctta gaacaataat cattttaagg ataacaagta aaggtctgaa agcatgaggg
26041 gctttatttg ccttcacctc atataagtct ttgattttga accaatgctt ttggatctca
26101 ttgttgatga tacttgaatt tactttgtag gagattttaa cttcatgctg atgatgtatc
26161 aaattcattt tatacaaagt ttaaagattt tttctggaag tgatacatgt caaattacat
26221 ttcctactgc agtatttgag cagggacagt catttttaa atgtttttgg ccgggtgtgg
26281 tggctcacgc ctgtaatctc agcacgttgg gaggccaagg cgggtggatc acctgaggtc
26341 agaagttcaa ggccagcctg gccaacatgg tgaaaccctg tctctacgaa aaatacaaaa
26401 aattggccag gcgtggtggt gggcgcctgt aatcccagcc actccggagg ctgaggcagg
26461 agaatcgctt gaacctgcga ggcggagatt gcagtcagcc aagatcaagc cattgtactc
26521 cagcctggac aacgagcgaa actctgtcta aaaaacacac acacacacac acacacacac
26581 aaaacaatgt tttcatgcct gtaaccctag cacattggga agccaagttt ggaggatcgc
26641 ttgaggccag gagttcaagg ctgcagtgag ctatgattgc accactatac tctagcctgg
26701 gagacagagt gagaccctgt ctctaaaaaa aagaaaaagt ttttgaacct taaaattact
26761 tctctttgtt tgaatttcta atcatcattc aaaagaacag ttaaaaaagg ttacttgttc
26821 ttgtgcaact acaaattaga ctggagtagg atattttaaa gagctgaatc acttttgqta
26881 ttttgttata aatgttttca tttgttatgt cccagtatat tcttattgga aaattcttgt
26941 tttgatctgc ctgaagaaaa tatctgtttt ctatataaag aaacatttaa aaataattgt
27001 aaagttagat ttaattgtaa aatataaaat cacaaaggaa tgtaccttat gaatgttgac
27061 attttatgaa attatgtaga ttcatattac tgttacaaga tagaattgaa tgcaaaaaga
27121 ccaaatcctc attaaaattt gaggaaaaca taagtgttat tatgtaattg aaataaaaac
27181 attttatagt tgtaaaaaaa attgcaagtg gaaccatctt aagttggggg acatctatat
27241 gtatttaaat ctagtctgac aatctttata tttgaaaaac agtttttta gagatagggt
27301 ctcacctatc actgaggctg gagtgcagtg gcacaatcaa gcttattgca gcctcaaaca
27361 cctgggctca agcaatcctc ctggctcagc ctcctgagta gctaggacta taggtgtgcc
27421 actacaactg gatggtgttt taatttttat tgtgtagaga cagggtcttg ctatgttgcc
27481 caggctggtc tcgaactcct gggttcaagt gattctccca cgttggtttc ccaaagtgtt
27541 gggactatag gcatgagcac cacagcctgc cctactctct atcttttaac tggatcattt
27601 actccattta gttttattgt aattactgat atactgatgc aataacatta ttctatcatg
27661 ttattctgtg ctatttgtcc tgacttnttc atgagttttt ccctcatctt tattgccttt
27721 tttggattga ttttttccct ttccattctt tgtttctcta ctagtttgga atttctggag
27781 tatcacctaa aagagtagag aaaggtgata tttctattta gcatgcatat ttgaactttt
27841 caacatgaaa ttaatgtctt tatttttccc catggaaaaa ttatcactgt tactccctct
27901 aaattatatt ctcttgtcat gtatttatct tttcttttaa ccccacaaga cataatagta
27961 ataagataat tactattatt attattttgt agaattaata tctttttttt tctttgagat
28021 ggagtctttc tctgttgccc aggctggagt gcagtggtgt gatcttggct tactgcaacc
28081 tctgcctcct gggtttaggt gattctcctg tttcagcctt ccgagtagct gggattacag
28141 gtgtgtgcca ccacgtccag ctaatttttg tatttttttgt agagatgggg tttcgccatg
28201 ttgggcagtc tggtctggaa ctcctgacgt caggtgatct gcccacctgg gcctcccaaa
28261 gtgttgggat tacaggcgtg agccactgtg tccagcctag agttaatatc tttacttacc
28321 tgtttacttc tgtatttgct cttcatttct ggttaaatat cagctgttat ctggattttt

FIG.8K

28381 ttcgttctgc cttgagtaaa tacttgaccc ttttccttca gtgagggtct atcaaggaca
28441 aactcagttt tgtgtttgaa atgtctttgt ctgaaatgtc tttattcatt gagaggattt
28501 ttttgtagac atagaattct gtggttactt tttctcagta tattgataat attcttgcct
28561 tatggcttct agtcttgtta cagagaagta agctctcagc caaattgtca ttactttgaa
28621 gttaattgtc tttttctttg gcgactgtta agatttcctc ttgtctttgt agctgtgcaa
28681 tatatctgtg atgtgtttaa gtgtggttcc tctttatttt atcccatggg cttctggagt
28741 ctgggaactg gtcttcaatc agttctagaa tttgactatc tttttaaaat attgcttctg
28801 acttatgtct cttttcttct ggaattctga atagatatta tgttacacta tttaatctat
28861 ctttcatgtc tctgaacctc tctttaatac tttccattta aaaatctctc tgtattatac
28921 tctggctatt tttgcagatc cagctctgtg ttcactaatt atcttctcag atgtatctaa
28981 ttgagtgtta tgtctgttca ttaaattttt actttcaatt attttatttt tgatttctat
29041 aaattctttt ttaaatatta gctctacatt ttagaattcc ttgatttctg acattttgga
29101 tccttccttt atttctttaa atatgttaca gatgtgtatt ttatattcta tgcaaatatc
29161 tgaaattttt cgtggatctc attttgtggt ctatttttt tgcactcaaa cgccttgatt
29221 tcctgtgtgt tttatgactt ttattttatt ttttattatg tatttattta ttcatttttt
29281 tttgagatag agtcttgctc tgttgcccag actgaagtgc agtggcacga tctcagctca
29341 ctgcaacatc caccttctgg gttcaagtga ttgtcccacc tcagcctccc gagtagctgg
29401 gattacaggt gcctgccact acacctggct aatttttgta tttttaatag agacagggct
29461 tagccatgtt ggccgggctc gtctcaaact cctgacctca ggtgatccac ctgccttagc
29521 ctctgaaagt gctgggatta tagccatgaa ctacctcgcc tggctgttgt gtgttttatg
29581 acttctaaat gagtgcatat actccttgga catttatata cgtcttcttt ttttatttta
29641 ttttatttga gacgagtctt gctctgtcgc taggctggag tgcagtggcg caatcttggc
29701 tcactgcaac ctccgactcc ttgttcaaag gattctcctg ccgcagcagc tagaattaca
29761 ggcacgtgcc accacgccca gctaattttt tgtgttttta gtagagatag ggtttctcac
29821 catgtaggcc aggatggtct cgatctcctg acttcatgat tcgcccacct cgacctccaa
29881 aagtgctggg attacaggcg tgagccattg cgcctggcct atagaattct tttaaggcct
29941 aagttaaatg tgtgtgccta cagagaacat acgtatttaa atttgccagg ttgcagaggg
30001 cactacctac ttaaacaaca ttacacgaaa ttcttagctt gagatttttg tattacccag
30061 gtagtatgaa ttcaggctgt aaactccctt gaggatcccc ttgaggatta tctaaaattt
30121 caggggagat tgtagttttc ctcttttagt cagtgttaag gtttgagaaa ggcattttcc
30181 ttgccatttc ctatggagtg gtacggtgtg ggtgaaggga gaggggctat ttccagttca
30241 acctgacact gacttttaag tcctttgggg tcccagctct agttggatgg tatattaaac
30301 tacatacctc ggatagaccc tggacattgt ctcctgtccc tgtgacccta taaactgtga
30361 aacaaaagct caagttcacc aagttccgca aatgccctca agttaaaact tgacttctgt
30421 ccaccttcct ttctgggttc ctactttcac atagtttttg tcttttgagt atttccaatt
30481 ctttttaagc tttggccaga agttttagtt gtctgtagtt agagtgggtg tctagtgtac
30541 cataccactg aaacagaagc ctgttacttt tacaataata aaaacatatc tatgtgggct
30601 ttttaaaaaa atttttaaat ttatttattt actttttttt ttgagatgga gtctcactct
30661 gtcgcccagg ctggagtgca gtggcatgat cttggctcac tgcaacctct gccacccggg
30721 ttcaagcgat tttcctgcct cagcctcctg agaagctgtg attacaggcg catgccaccg
30781 tgcctggcta aattttgtat ttttaggaga gacaggtttc accatgttgg tcaggctggt
30841 ctcaaactcc tgagctcagg tgatctgccc accttggcct cccaaagtgc tgggattaca
30901 agcatgagcc actgcacctg gccaaaccta tctatgtttt agtttttttat gattatttta

FIG.8L

30961 tattatccct tccccacaca tgaaataact tctttccaag tgacttatga agttgtcaac
31021 ttttcataaa gccttggaac aaagtgggca gaaaaattat aaataaaaag tcttgtctag
31081 gacagtacag gtctgtctta ttcctaaatt agatcagaat cgggttatgc cggtttttta
31141 taacatacca ttataattgg gtatgttaaa gaatgtatta gagatgcatt agaagagcga
31201 cctcattata agcctcttca cccatggatt ccaaggatat cttacaataa acctctggga
31261 taccttacgc tacagagcaa ctaaagtcca gctttagagc acaagggaaa atgcaggata
31321 ttgggtcctg gaaactacaa aaaccatcaa actctacttt agggctaaaa ctttctttta
31381 atcaatctgt catatttatt gataaattag aaaatgtggc tgggtgcagt ggctcacacc
31441 tgtaatccta gcagtttggg aggccgaggt gggtggatca cgaggtcagg agatggagac
31501 cattctggct aacacagtga aacccgtctc tactaaaaat acaaaaatta gccgggcgtg
31561 gtggtgggcg cctgtagtcc cagctactca ggaggcggag gcaggagaat ggcttgaacc
31621 cgggagacgg aggttgcagt gagccgagat tgccccactg cactccagcc tgggtgacag
31681 agggagactc catctcaaaa aaaaaaaaaa aaaagtacag gttcaaattt cattaaattt
31741 tttttccatg ccccttctct ggtggtagga tgatagggga tgagtaaaat atcaaagaca
31801 actaaaaatt cctaaactag atcctcgggt cccaaagaag tgttaaccat ggagtgcccc
31861 accttctgag gttggtgctt cctggcacag gctcttggaa cattctggat tgtctgacat
31921 tccttttact cgctttagaa cctaaagatg ttgctggggg aaaagggagc tagggagagg
31981 gaaggggaag gatgtgggat aaggcataaa ctatgcttgt gggaaaaaaa caaccagtaa
32041 tttccttgat ggggtttttg cttgacttta aaatgcagta agcttttagg aagcttgatg
32101 gagcctgtgt ttctaccatt agtgctgcat ttgtttggct taagatttca ttaagcttct
32161 taaaatggag atacttctgg aactcaaatg gctactgagc agggaatggc aaatcagtga
32221 cactgtagga agtggaggga ttttggcaaa ctagagaaca caccttccac aggggcagcc
32281 actgctctgc tgggcccgct gttcatctgc agggacgtgc atcttgggat tatttccaaa
32341 gtcagaactc agcattttta tgagaaatgt catgattttt aaggtcttaa caagttatcc
32401 cacattaaaa aaataataat aaaccaggcc gggtgcagtg gctcacgcct gtaatcccaa
32461 cattttggga ggctgaggtg ggtggatcat gaggtcaggc gttcgagacc attatggcca
32521 acatggtgaa acactgtctc tacttaaaat acaaaaatta gctgggcatg gtggccggcg
32581 cctgtaatcc cagctactag ggaggctgag gcaggagaat cgcttgaacc tgggaggcgg
32641 aggaggttgc agtgaactga gatcgtaccc agcctgggca acagtgcgag gttccgtctc
32701 aaaaaaaacc aaaaaacaca aaaacaaaaa acgacagaga aggccaaaca aaacacatct
32761 gtgggctgga tgccgccatg cccaccggtt tgcgaccttt gtgttggact cttctgttca
32821 ccagacaccc tgccctgcga gaatgtatct catcctttgc tggagcaggt ttgcaggcac
32881 agtggagaga ggagagaaga aatgaaggga cacttatgca gaaccatgag tggccagaga
32941 ggaggagaag gagggtgaga ggagcaaaga agccatgaca acttcataat tctgagtgga
33001 ctgggcagtg gccagaaatt ctggtggtgg atatgctgcc tttccaacag gtgaatatga
33061 aagaataagt caaaccctgt tcaggacgct gttaattcca aatgtgaact ttttgagtca
33121 ttctttccat gtggaattca aaggagaatg taaacaaatt ttcaggaggg acgtgcaata
33181 tccctgaaag ataacaaagt tcgtaacact tatttacata caacattctc tagttattga
33241 ttaaacagat ctctacagac ttgcatgagg caacatttct taggcttgtt tgctacaata
33301 tctttaaaaa tacttgatta cacatcactt tagcttattt agatggactt ttcaccaagc
33361 tctgaactgg gatttcattt tgttgcattc atcctgctca cgagacacag gtaggcagca
33421 aatgagatta tccctccagt ccccatggat tggaaatgtt ccccttctt tatgagctca
33481 ctgcagtatc tccttctccc tttccccaaa ggacagcctt tcctgcctca gggaagaaga

FIG.8M

33541 gagagacaga ctacagtgat ggagacccac tagatgtgca caagaggctg ccatccagtg
33601 ctggagagga ccgagccgtg atgctggggt ttgccatgat gggcttctca gtcctaatgt
33661 tcttcttgct cggaacaacc attctaaagc cttttatgct caggtaagaa acagaggaag
33721 gaaatctaga gtttccaatt cagataagat ccaggcacag cggtaaagga ggagaggctg
33781 gctttggttt ctcaatctgt gactctctgc catggtctag aaaaagaaaa atacaattcc
33841 tcttccgctg tcagtgctgg gggcagctgg gtgtggagga agaggtggcg agaaggtcaa
33901 agatatttcc ctgcactgcc tcctcccatc tcataatcta tatatacctc caaacacgta
33961 atccacaaat tatagtttct tcatttaggt cataaatcct ccccttaaaa tgttggtatt
34021 ccaagagaga gttaaacctt gtatgtgagc acaataaaag ttttttgagt ctgaattttt
34081 tgagtttgac agtgtctacc tggcacatag tagttgctca atacatatta gtttccttcc
34141 ttttaattag gttctttatt caatatatgt agtgatacag ttgacctttg aacaacatgg
34201 gtttgaactt cgggaatcca cttataaatg gattttcttc tgtgcctgcc acccctgaga
34261 cagtaagatc aatccctcct ctttctcctc ctcctcagcc tactcaacat gaagaggaca
34321 gggagaggac ctttatgatg atccacttcc atttagtaaa tagtaaacat gttttctctt
34381 ccttatgatt ttttttcttc aatttttgtt ttaagttccg gggtacatgt acagaatgtg
34441 caggtttgtt acaaaggtaa acgtgtgcca tggtggtttg cagcacagat caacccatca
34501 cctgggtatt aagcccagca tgcattagct attcttcctg atgctctccc tccccctact
34561 ccactgagag gccccagtgt gtgttgctcc cctctaggtg tccatgtgtt ctcatcattc
34621 agctcccact tttaagtgag aacattcagt gtttggttct ctgtttctgc attagtttgc
34681 tgaggataat ggcttccagc ttcatttcat ctatgtcccc gcaaatgaca tgatcttgtt
34741 cttccttatg attttctttc cttttctttt ttctgagacg gagtcttgct ctgtcaccca
34801 ggctgaagtg cagtggcacg atcttggctc actgcaacct ctgcctcccg aattcaagca
34861 gttctcctgc ctcagcctcc caagtagctg ggaccacagg tgtgtgccac catacttggc
34921 taatttttaa attgttggta gagatgggat ccccctatgt tgcccaggct ggtcgtgaac
34981 tcctaagctc aagtcatcct tctaccttgg tctcccaaag tgctgggatt acaggcatga
35041 gccaccatgc ctgaccagta gttaaatttt tgaggagtca aaagttatat gcaaattttc
35101 gactgcaggt ttgggggtgt ggtgttggca tccctaaccc ctgaattgtt caagggtcaa
35161 ctttagttgc tacttattaa gtatatgctg tatgctcaat acatactata ttttatccta
35221 cttaaaccta cagcagccca tgatggaagt gttattttag ttttcatttt ataaatgaga
35281 aagctgaggc tcagaaaggc caagtaactt gcccaagttc acatagccgg taaataatat
35341 gcctgtcatt ctgacgtaag acttgcttct taatacctaa atgatgtttg ctgaaatgag
35401 gggaatgggg gttctgatta gggaagggga ggggctattt aattttgtag gatggcccac
35461 acaacatgtg gtactctggg gccaggtcag ttcagggtaa aaggaaggca aggggtgctt
35521 tttggacatt gctttatttt tggacagccc ttttttttt ggacagccaa agagcaacag
35581 gaggcccagg gggagtggaa gaggaaggcc taggagttca gtgagggaaa ggggtcataa
35641 aaaaggaaga gagtggccta cttctgttat ttatgattca tctcctgcca ttcctaggtt
35701 accattgtta ggggaaagat gtagaagtca ccatttacta tagcacacag tgccactgtt
35761 ggaagaattc cgcggatcct ctccctatag tgagtcgtat tagcggccgc aaatttatta
35821 gagcaatata gtcctacaat gtcaagctcg accgatgccc ttgagagcct tcaacccagt
35881 cagctccttc cggtgggcgc ggggcatgac tattggcgcg ccggatcgat ccttaattaa
35941 gtctactaga taacttcgta taatgtatac tatacgaagt tattatctat gtcgggtgcg
36001 gagaaagagg taatgaaatg tgccccgctt acgcagggca tccatttatt actcaaccgt
36061 aaccgatttt gccaggttac gcggctgcag gcatgcaagc tttggcgcgc cgtcgaccaa

FIG.8N

36121 ttctcatgtt tgacagctta tcatcgaatt tctgccattc atccgcttat tatcacttat
36181 tcaggcgtag caaccaggcg tttaagggca ccaataactg ccttaaaaaa attacgcccc
36241 gccctgccac tcatcgcagt actgttgtaa ttcattaagc attctgccga catggaagcc
36301 atcacaaacg gcatgatgaa cctgaatcgc cagcggcatc agcaccttgt cgccttgcgt
36361 ataatatttg cccatggtga aaacgggggc gaagaagttg tccatattgg ccacgtttaa
36421 atcaaaactg gtgaaactca cccagggatt ggctgagacg aaaaacatat tctcaataaa
36481 ccctttaggg aaataggcca ggttttcacc gtaacacgcc acatcttgcg aatatatgtg
36541 tagaaactgc cggaaatcgt cgtggtattc actccagagc gatgaaaacg tttcagtttg
36601 ctcatggaaa acggtgtaac aagggtgaac actatcccat atcaccagct caccgtcttt
36661 cattgccata cggaactccg gatgagcatt catcaggcgg gcaagaatgt gaataaaggc
36721 cggataaaac ttgtgcttat ttttctttac ggtctttaaa aaggccgtaa tatccagctg
36781 aacggtctgg ttataggtac attgagcaac tgactgaaat gcctcaaaat gttctttacg
36841 atgccattgg gatatatcaa cggtggtata tccagtgatt tttttctcca ttttagcttc
36901 cttagctcct gaaaatctcg ataactcaaa aaatacgccc ggtagtgatc ttatttcatt
36961 atggtgaaag ttggaacctc ttacgtgccg atcaacgtct cattttcgcc aaaagttggc
37021 ccagggcttc ccggtatcaa cagggacacc aggatttatt tattctgcga agtgatcttc
37081 cgtcacaggt atttattcgc gataagctca tggagcggcg taaccgtcgc acaggaagga
37141 cagagaaagc gcggatctgg gaagtgacgg acagaacggt caggacctgg attggggagg
37201 cggttgccgc cgctgctgct gacggtgtga cgttctctgt tccggtcaca ccacatacgt
37261 tccgccattc ctatgcgatg cacatgctgt atgccggtat accgctgaaa gttctgcaaa
37321 gcctgatggg acataagtcc atcagttcaa cggaagtcta cacgaaggtt tttgcgctgg
37381 atgtggctgc ccggcaccgg gtgcagtttg cgatgccgga gtctgatgcg gttgcgatgc
37441 tgaaacaatt atcctgagaa taaatgcctt ggcctttata tggaaatgtg gaactgagtg
37501 gatatgctgt ttttgtctgt taaacagaga agctggctgt tatccactga gaagcgaacg
37561 aaacagtcgg gaaaatctcc cattatcgta gagatccgca ttattaatct caggagcctg
37621 tgtagcgttt ataggaagta gtgttctgtc atgatgcctg caagcggtaa cgaaaacgat
37681 ttgaatatgc cttcaggaac aatagaaatc ttcgtgcggt gttacgttga agtggagcgg
37741 attatgtcag caatggacag aacaacctaa tgaacacaga accatgatgt ggtctgtcct
37801 tttacagcca gtagtgctcg ccgcagttga gcgacagggc gaagccctcg agtgagcgag
37861 gaagcaccag ggaacagcac ttatatattc tgcttacaca cgatgcctga aaaaacttcc
37921 cttggggtta tccacttatc cacggggata tttttataat tatttttttt atagttttta
37981 gatcttcttt tttagagcgc cttgtaggcc tttatccatg ctggttctag agaaggtgtt
38041 gtgacaaatt gcccttttcag tgtgacaaat caccctcaaa tgacagtcct gtctgtgaca
38101 aattgccctt aaccctgtga caaattgccc tcagaagaag ctgtttttttc acaaagttat
38161 ccctgcttat tgactctttt ttatttagtg tgacaatcta aaaacttgtc acacttcaca
38221 tggatctgtc atggcggaaa cagcggttat caatcacaag aaacgtaaaa atagcccgcg
38281 aatcgtccag tcaaacgacc tcactgaggc ggcatatagt ctctcccggg atcaaaaacg
38341 tatgctgtat ctgttcgttg accagatcag aaaatctgat ggcaccctac aggaacatga
38401 cggtatctgc gagatccatg ttgctaaata tgctgaaata ttcggattga cctctgcgga
38461 agccagtaag gatatacggc aggcattgaa gagtttcgcg gggaaggaag tggtttttta
38521 tcgccctgaa gaggatgccg gcgatgaaaa aggctatgaa tcttttcctt ggtttatcaa
38581 acgtgcgcac agtccatcca gagggcttta cagtgtacat atcaacccat atctcattcc
38641 cttctttatc gggttacaga accggtttac gcagtttcgg cttagtgaaa caaaagaaat

FIG.80

38701 caccaatccg tatgccatgc gtttatacga atccctgtgt cagtatcgta agccggatgg
38761 ctcaggcatc gtctctctga aaatcgactg gatcatagag cgttaccagc tgcctcaaag
38821 ttaccagcgt atgcctgact tccgccgccg cttcctgcag gtctgtgtta atgagatcaa
38881 cagcagaact ccaatgcgcc tctcatacat tgagaaaaag aaaggccgcc agacgactca
38941 tatcgtattt tccttccgcg atatcacttc catgacgaca ggatagtctg agggttatct
39001 gtcacagatt tgagggtggt tcgtcacatt tgttctgacc tactgagggt aatttgtcac
39061 agttttgctg tttccttcag cctgcatgga ttttctcata ctttttgaac tgtaattttt
39121 aaggaagcca aatttgaggg cagtttgtca cagttgattt ccttctcttt cccttcgtca
39181 tgtgacctga tatcgggggt tagttcgtca tcattgatga gggttgatta tcacagttta
39241 ttactctgaa ttggctatcc gcgtgtgtac ctctacctgg agtttttccc acggtggata
39301 tttcttcttg cgctgagcgt aagagctatc tgacagaaca gttcttcttt gcttcctcgc
39361 cagttcgctc gctatgctcg gttacacggc tgcggcgagc gctagtgata ataagtgact
39421 gaggtatgtg ctcttcttat ctccttttgt agtgttgctc ttattttaaa caactttgcg
39481 gttttttgat gactttgcga ttttgttgtt gctttgcagt aaattgcaag atttaataaa
39541 aaaacgcaaa gcaatgatta aaggatgttc agaatgaaac tcatggaaac acttaaccag
39601 tgcataaacg ctggtcatga aatgacgaag gctatcgcca ttgcacagtt taatgatgac
39661 agcccggaag cgaggaaaat aacccggcgc tggagaatag gtgaagcagc ggatttagtt
39721 ggggtttctt ctcaggctat cagagatgcc gagaaagcag ggcgactacc gcacccggat
39781 atggaaattc gaggacgggt tgagcaacgt gttggttata caattgaaca aattaatcat
39841 atgcgtgatg tgtttggtac gcgattgcga cgtgctgaag acgtatttcc accggtgatc
39901 ggggttgctg cccataaagg tggcgtttac aaaacctcag tttctgttca tcttgctcag
39961 gatctggctc tgaaggggct acgtgttttg ctcgtggaag gtaacgaccc ccagggaaca
40021 gcctcaatgt atcacggatg ggtaccagat cttcatattc atgcagaaga cactctcctg
40081 cctttctatc ttggggaaaa ggacgatgtc acttatgcaa taaagcccac ttgctggccg
40141 gggcttgaca ttattccttc ctgtctggct ctgcaccgta ttgaaactga gttaatgggc
40201 aaatttgatg aaggtaaact gcccaccgat ccacacctga tgctccgact ggccattgaa
40261 actgttgctc atgactatga tgtcatagtt attgacagcg cgcctaacct gggtatcggc
40321 acgattaatg tcgtatgtgc tgctgatgtg ctgattgttc ccacgcctgc tgagttgttt
40381 gactacacct ccgcactgca gtttttcgat atgcttcgtq atctgctcaa gaacgttgat
40441 cttaaagggt tcgagcctga tgtacgnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnngac
40501 agtctatctg ttagttatat tctagccctt tatagcttgc tggtctcaag atgtagaatt
40561 gctgaaagat gtaattcaga ctgtttggaa agaagacaag tcctccctcc ttcctgggtt
40621 cactgtgaac cacggaggtt tctacgatac atggtctaac cactgaaaat ttactgtcct
40681 tactgagata ttttagaagt gtaaaataca gactgtattt caaagacagc atgaaaaagc
40741 cagcttttgt agcaggcacc aagcttgttt tactttatac aggctcccta attcctctct
40801 gccacttcaa ccttcaactg atatccctgg cagaaggatg aaaaatcaaa agggcaagag
40861 atagaacatt tgtttcaggt attgggacaa ctccaagtat taagataaca gattcatttc
40921 atgctgtttc atttaatcaa tgtttattca tggcaaattt tatttttat tatttttatt
40981 ttttatttta atttaagtgg gtacatagta gttttatgta tttatgggtt acgtgagata
41041 ttttgataca ggcatgcaat aatcacatca gggtaaatgg ggtgtccatc atctcaagta
41101 tttatccttg tgtcacaaac aatccaatta tactcttagt tgtttaaaaa tgcggggagt
41161 ggagggcaag tggagggaga gcattaggac aaatacctaa tgcatgcggg gcttaaaacc
41221 tatgtgatgg gttgacaggt gcagcaaacc accatggcac atgtatacct atgtaacaaa

FIG.8P

41281 cctgcacgtt ctgcacatgt atcccgtaat gtaaagtaaa ataaaatagc ataaaatcaa
41341 ataaaggaat cactaatgaa acctacattt gcaaaataaa ttataaatat tgaaaagaaa
41401 taaaaagtac aaaaaattat ttttgtctat agtcactcta ttgtgctatc aaatactagt
41461 cttattcatt ctttttgttt tttgtaccca ctaaccatcc cactccccgc cctacacccc
41521 ccaacaaccc gccccatact cttccaagcc tctggtaacc gtccttctac tatctccatg
41581 agtcgaattg ttttaatttt taacttccac aaataagtga gaatatgtga agtttgtctt
41641 tctgtacctg gcttaaattt cacttaacat aatgacctcc agttccatcc atgttattgc
41701 aaatgactgg acctcatttt cttaatggct gaatagtact ccattgtgta tatgtaccac
41761 attaaaaaaa atcaatttgt tagttgatgg attgctttca aatcttggct attgcgaata
41821 gtgctgcaat agctaagatt tggatatctt tgggagtgta gatatctttg ggatatcttt
41881 taatcttttc ttttcttttt gagacagagt ctcgctctgt tgcccaggct ggagtgtagt
41941 ggcgcaatct tggctcactg caacctctgc ctcccgggtt caaacaattc tcctgcttca
42001 gcctcctgag tagcttggat tacaggcact caccaccatg cccagataat ttttgtattt
42061 ttagtagaga cggggtttca ccatgttggc caggctggtc tcaaactcct gacctcagat
42121 gatccaccca tctcggcctc ccaaagtgct gggattacag gcgtgagcca cctcgcccag
42181 cctgattttt tttcttttga gtatatacct acctctattt tcagtttttt gaggcacctc
42241 caaactgttc tccatagtga ttgtactaat ttacattccc actaacagtg tacaagggtt
42301 ccctttttctc cctattcttg ctagcatttg ttattgcttg acttttggat aaaagccatt
42361 ttaactgggg tgagatgata tgtcattgta gttttgattt gcatttctct gatgatcaat
42421 gacaatgcct ttttgtatgc ttgtttccca cttgtatgtc ttcctttgag aaatgtctat
42481 gcagatcttt tgcccattta aaaaactgga ttattatatt ttttcctata gagttgtttg
42541 agctcctttt atattctgat tattaacccc ttgtcagatg ggtagtttgc aattattttc
42601 taccactctg tggtttgtct cttcactttg ttgattcttt cttttgctat gcagaaagtt
42661 tttaatttga tatgatccta tgtgttcaca tttgctttgg ttgcctgtgc ttatgaggtt
42721 ttactcaagg aatctttgcc tactctggga cttactgttt tcttttacta gtttcatagt
42781 tcgaggtctt aaatttcagt ctttaatcca ttttgatttg atttttgtat aaggctagag
42841 ataacaggtc tagttcactc ttttgcatat ggatatccag ttttcccagc atcatttatt
42901 gaagagactg tcgtttcccc aatttatgct cttggtaact ttgtcaaaaa tgaattcact
42961 gcagatgtat gcatttacct ctgggttctc tattctgttc cactgatgta tgtgtctgtt
43021 tttatgccag taccttgctg ttttggttac tgtagtttta tagtataagt ttaagtcaga
43081 taaagtgatt tctcccattt tgttcttttt gctcagggta gctttggctc ttctgggtct
43141 ttcgtggttc caaatgaatt ttagaaattt ttttctattt ctgtgaagaa tgtcattggt
43201 attttgatag gcattgcatt aaatctgtag atcgctttga gtaatatgga cattttaaca
43261 ttattgatat ttccaatcca tgaacatgta atgtctttcc ttttttgtgg cttcttcaat
43321 ttcttgcatt aatgttttat agttttcatt ttagatatca ttcacttcgt tagttaattc
43381 ctaggtattt aattttattt gtagctattg taaatgggat cttttaaaat ttctttttca
43441 gattgttcac tgttagcata tagaaatgct actgatgttt gtatgttgaa tttgtatcct
43501 gcaactttac tgagtttgtt tatcagttct tatagttttc ttgatggtat ctttagtatt
43561 tcccaaatat aagatcatat ctgaaaacta gaataatttg acttcttcct atccaatttg
43621 gatgcccttt atttctttct cttgtatgat tgctcaagct aggactttca gtattatgtt
43681 gaataacagt ggtgtaagtg ggcatccttg ttgttttcca gatcttagag aaaaggcttt
43741 caatttttcc ccattcagta tgatactaac tctggatctg ttgtatatgc tttttattat
43801 gttgaggtat gttccttctg taaccagttt tgtgaggttt ttgttatgaa gggctgttga

FIG.8Q

43861 attttatcaa gtgctttttc agcatcaatt gaaatgatca tatagttttg tctttcattc
43921 tgttgatatg atgtattaca ttaattgatt tgcatatgtt gaactatctt tgcatcccta
43981 ggctaaatct cacttcgtca taatgaatga tgagtgattg agttagtgaa tattgatgag
44041 ttatcgaata ttgagtttga tttggtagta tttgagaatt tttgcatcaa tattcatcag
44101 aaatattggt gtatagtttt atttttattg atgtgtcttt gtctagtctt agtatcaaga
44161 taatactggc ctcatagaat gagtctggaa gtattccctc ctttctttgt gtttttgaat
44221 agtttgagta ggattggtat agtttttttt tatttaattt gtttatttgt cttgagacac
44281 ggtgtcactc tgtcaccaca ggctggagta cagtggtgtg atcttggctt accatagcct
44341 ctgcctcgcc tcccaggctc aagcgattct ctaataatat ttgctttata tatctgggtg
44401 ctccagtgtt tggtgcatat atatttatca aattgttata tcctcttgct gaattgaccc
44461 ctttatcatt atatagtgac cttcttgttc tcttcttata gtttttgtct tgaaatctat
44521 ttgtctgatt tatccaattt atttatctga ctcctgctct ttttttggtt tccatttgca
44581 tggaatattt ttttccatcc cttattttca gtttactgtg tattcttgga caagttactt
44641 tctgagcctc aatttcctca tctgatagtt ggtgtctgtt aggatctatc ttacaggact
44701 gttttaagga ttaaatgaga caatgcacag tgcctggggc atgatgagat atggtaatta
44761 ggaaataatt actcaggcca ggcacggtgg ctcatgccta taattccaac attttgggag
44821 gctatggtgg gaggatcgct taacaccagc ctgggcaata tagtcacacc cagtctctcc
44881 aaagaaagaa agaaagaaag aaaaaaatta gcccactatt gtggtcacat gactgtagtc
44941 ccagctactc aggaggctga gatgggagga tcacttgagc ctggaaggtt gagggtgcag
45001 tgagccatga ttgtgctact gcgctccagc ttgggcaaca acatgagaac ttatttcaaa
45061 atagctaaca aatggtagtt attattgtta catttattta tttagaatta ttattcaatt
45121 attattatgt aataatcaac aaaaattatc ctttttttgta gcatcttttt atctcttctc
45181 tgatttcttt tttgaacaaa tttaaaaaca tattataata ttaaaaaacg gagaccaggt
45241 ctccctgtgt tacccaggct gtcctcaaac tcctgggctc gagcaatcct tccgccacag
45301 ctttccaaag tgctgcggtt acaggcgtga gccactgtgc ctggccttgc attatttctg
45361 ttagggttat ttgtttattt gttatctgtg taatttattt actttgatgt ttaaagcaaa
45421 gaattacaac tcagtgaagg agatatcctc acatgtaact tgcaactcta aatatgacct
45481 tatctaagag ggtattagtt tcaattatgc tgcccctatg ggtcattgaa gtgagactct
45541 tctgtgtgtc ccataagagt gcactaaggc aactgaagtc atttgatgtc ctcagccccc
45601 ttggcaagca cagaggtgct caaaagactg atgaacttgg cactaagatt tttcttccag
45661 aagacaggga gaaatcacca aatggttttt tttttaaagc aaactaaata tttagccacc
45721 ttttgggttc actttttttt ttctttttct tttttactag tcattgactt gaagctcatg
45781 gttttactct ttaacaccag ccagtcaact gcaggttcct gggacaaaaa gcaaaaggaa
45841 taatgatccc ttgtggattc ctgagtcacc tgctttgtgg gctgtgcagc gtctgtggtc
45901 agcaccacac cggctacctc actattgctc agggtttgtg ctttgggagc tagcctggaa
45961 gctaagaact ctcactttca cctggtatta actactttgt ttctttttttt ttttttttt
46021 tgagacggag tctcgctctt gtcacccagg ctggagtgca gtggcacgat ctcagctcac
46081 tgcaacctct gcctcctggg ttcaagcgat tctcctgcct cagcctcccg agtagctggg
46141 attaaaggct cctgccacca ccccgagcta attttttttta tttttagtag agatggagtt
46201 tcatcatgtt gggcaggctg gcctcgaact tctgacctcc ggtgatcctc ccaccttggc
46261 ctcctgaagt gctgggatta caggcgtgag ccacattgcc cagcctggta ttaactactt
46321 tctaaaggcg cttcctgttg cacaagaatg ctgtttgcag gaatgcctgt agacacatga
46381 accctgggcc tgctgctccc ttaatttgag ggaagctatg gatgtcaagc agaatagagc

FIG.8R

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46441 tattttgtta cttgaccagg tttccataac tttttgcaca tacctttatt cctgcacctg
46501 tcccacataa ttatcccttt atagctcttt caccagcagg aatataaatg tcatggcagg
46561 gccaccatgt tgcaaactcc ctagggtcca tcctgcagtt actagcttgt gtgaatggta
46621 cttcttggag gtaccattgc aatgcacagc ctgagcagcc ctctgcagta agtccaactg
46681 tggatggggc aaaatgagtg gttagcagaa ttggcctcat acatctttta ttctggcatt
46741 gagtatgtgg taggcactca agaaatattt ttaaatgaat gacattggtc ttcccattta
46801 ttttgagtta atttgaccca gcttaatcag tggatctttt caactagtta attgtcctac
46861 ccttccaccc aggaaggtgt ttcagtttta atagattttt gttattggtg ttttttgca
46921 cattcagcaa aacctctgga aagaggagtt catatcagcc cctcatgcag agggagctgt
46981 gagtattcag gctgcttggt gctgctgcgg tctttccagc agaggtggct ggtcctagag
47041 tggtggtttg gtccattctt gacctatgat gatgctggga aataactcag tgatgtggtg
47101 tcatttttt atctcacaga tgtggctctg gagaagagat ttaagtttat atcttcagaa
47161 catagcatca atgagtttgg gtcatagctg cagccaactc agccagaaac agatacacag
47221 tatgcaacca agtgggaata aattcaaccc attttcctaa tttggaatat agagcctgag
47281 gggtgaagtt gtgggtggat tatctgtcct gggttcttcc tatttcctga atttatactt
47341 tagtgaaggg aaattttgct aactgttaat gtgccagaat gggaaagccc cctacctagg
47401 ccctcacctc tgaatttttc cagttcaagt ggacaagtag tttttgagca ctcactatgt
47461 gtcaggctca gggtgacctt tgcgatttcc acttccttct gatcttttca cctaagcctg
47521 tgggacttgt gttctgggag aaaattagaa gttacatcat ccttatggtt ctgaaaaaat
47581 tgagctcccc agacttgggt tcgcctgatc tgcttctcaa atgttaaaga ggaacataaa
47641 gccatgtaga aaatcctttt cttttgacaa agcctggctt tcaaattcta ctgttgctgc
47701 agtcaccaaa attatacatt tgaatgtcag agattgaatg ttgacatcct tattgcaaaa
47761 atcctatccc cacttctggc ttagctgccc cagagagctc tgtgtctcgg gctgactaga
47821 gaaaagacca cacagttaga gatgcaaaaa aggaaatgcc attaacatat ttcagggtga
47881 atatttcttt gcatttggca ctttgcaatc tagtccaggc ttaagatttg taggatttct
47941 tttgctgttt ccctttttt acttgtcagg gaaacaaggc ataaatattt cagcttggac
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FIG.8S

ISOLATED NUCLEIC ACID MOLECULE(S) ENCODING A HUMAN CALCIUM SENSITIVE POTASSIUM CHANNEL SUBUNIT PROTEIN DESIGNATED BETA2, ENCODED PROTEINS, AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

[0002] Not applicable.

REFERENCE TO MICROFICHE APPENDIX

[0003] Not applicable.

FIELD OF THE INVENTION

[0004] The present invention is directed to novel human DNA sequences encoding subunits of calcium sensitive potassium channels.

BACKGROUND OF THE INVENTION

[0005] Voltage-gated potassium channels form transmembrane pores that open or close in response to changes in cell membrane potential and selectively allow potassium ions to pass through the membrane. Voltage-gated potassium channels have been found in cells traditionally considered both excitable (e.g., neurons, myocytes, secretory cells) and non-excitable (e.g., T-cells, osteoclasts) and have been shown to maintain cell membrane potential and control the repolarization of action potentials in such cells. Following depolarization, voltage-gated potassium channels open, allowing potassium efflux and thus membrane repolarization. This behavior has made voltage-gated potassium channels important targets for drug discovery in connection with a variety of diseases. As a result, many voltage-gated potassium channels have been identified and many cloned. They are distinguishable by differences in primary structure and tissue-specific patterns of expression, as well as by electrophysiological and pharmacological properties. For reviews of voltage-gated potassium channels see Robertson, 1997, Trends Pharmacol. Sci. 18:474-483; January & January, 1997, J. Physiol. 505:267-282; Catterall, 1995, Ann. Rev. Biochem. 64:493-531.

[0006] Many functional voltage-gated potassium channels are believed to be tetramers of four α subunits, each of which contains six transmembrane spanning segments. The α subunits making up a tetramer may be the same (in the case of homotetramers) or may be different (in the case of heterotetramers). The membrane-spanning α subunits making up the tetramers may sometimes be associated with additional, β subunits, which may alter the behavior of the α subunits.

[0007] A particular type of voltage-gated potassium channel is the voltage-gated and calcium sensitive potassium channel, also known as the calcium sensitive potassium channel. Calcium sensitive potassium channels are present in a wide variety of cells and are unique among voltage-gated potassium channels because their activity is regulated not only by changes in membrane potential but also by intracellular calcium concentration. Plasma membrane depolarization and increases in cytoplasmic calcium concentration both raise the open probability of calcium sensitive potassium channels. Therefore, calcium sensitive potassium channels can serve as a link between cellular processes involving increases in intracellular calcium and membrane excitability. Calcium sensitive potassium channels are believed to play a negative feedback role by terminating signaling events involving an increase in intracellular calcium, e.g., glucose mediated insulin release, blood vessel muscle tone, bronchial airway smooth muscle tone, and regulation of intraocular pressure. (Tanaka et al., 1997, J. Physiol. 502:545-557; Kaczorowski et al., 1996, J. Bioenerg. Biomem. 28:255-267; Vergara et al., 1998, Curr. Opin. Neurobiol. 8:321-329).

[0008] Certain calcium sensitive potassium channels have been isolated and studied. Functional calcium sensitive potassium channels are composed of α subunits that may be associated with smaller β subunits. The α subunit is believed to form the channel pore while a previously described β subunit increases the calcium sensitivity of the channel and makes the channel susceptible to regulation by certain substances, e.g., dehydrosoyasaponin (McManus et al., 1995, Neuron 14:645-650). The calcium sensitive potassium channel from bovine tracheal smooth muscle was purified and shown to be composed of an ~130 kDa α subunit and a 31 kDa β subunit (Garcia-Calvo et al., 1994, J. Biol. Chem. 269:676-682). Tseng-Crank et al. (1994, Neuron 13:1315-1330) cloned nine related calcium sensitive potassium channel α subunits from human brain. These α subunits are thought to be splice variants derived from a single gene, the h-slo gene (Tseng-Crank et al., 1994, Neuron 13:1315-1330). Knauss et al., 1994, J. Biol. Chem. 269:17274-17278 purified and cloned a β subunit of a calcium sensitive potassium channel from tracheal smooth muscle.

[0009] In most cells, the opening of calcium sensitive potassium channels results in the generation of non-inactivating, hyperpolarizing potassium currents. However, in certain cells (e.g., chromaffin cells of the adrenal gland and hippocampal neurons), the currents are inactivating. Following the discovery of the invention described herein, Wallner et al., 1999, Proc. Natl. Acad. Sci. USA 96:4137-4132 disclosed the existence of the human β2 calcium sensitive potassium channel subunit that, when combined with the α subunit, formed inactivating calcium sensitive potassium channels. The ability to confer inactivation was ascribed to the N-terminal 19 amino acids of the β2 subunit.

[0010] U.S. Pat. No. 5,776,734 is directed to nucleic acids encoding the bovine and human β1 subunit of the calcium sensitive potassium channel. U.S. Pat. No. 5,637,470 is directed to methods of identifying compounds that modulate the activity of calcium sensitive potassium channels.

SUMMARY OF THE INVENTION

[0011] The present invention is directed to novel human DNA sequences encoding β subunits of calcium sensitive potassium channels. The present invention includes DNAs that encode the β subunits β2, β3a, β3b, β3c, and β3d of human calcium sensitive potassium channels. The DNAs comprise the nucleotide sequences shown in SEQ. ID. NO.:1 (β2), SEQ. ID. NO.:3 (β3a), SEQ. ID. NO.:5 (β3b), SEQ. ID. NO.:7 (β3c), and SEQ. ID. NO.:9 (β3d). Also

provided are proteins encoded by the novel DNA sequences. The proteins comprise the deduced amino acid sequences shown in SEQ. ID. NO.:2 (β2), SEQ. ID. NO.:4 (β3a), SEQ. ID. NO.:6 (β3b), SEQ. ID. NO.:8 (β3c), and SEQ. ID. NO.:10 (β3d). Methods of expressing the novel subunit proteins in recombinant systems are provided as well as methods of identifying activators and inhibitors of potassium channels comprising the subunits.

[0012] The present invention also includes a genomic DNA fragment containing the 5' portions of the β3a, β3b, β3c, and β3d subunits, as well as the 5' portion of the core portion of the β3 subunits. This genomic DNA fragment contains promoter elements for the subunits. Methods of screening for compounds which affect transcription of the gene encoding the β3a, β3b, β3c, and β3d subunits are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **FIG. 1A** shows a DNA sequence encoding the β2 subunit of the human calcium sensitive potassium channel (SEQ. ID. NO.:1). The start ATG codon is at position 271-273; the stop codon is at position 976-978. **FIG. 1B** shows the deduced amino acid sequence (SEQ. ID. NO.:2) of the β2 subunit.

[0014] **FIG. 2A** shows a DNA sequence encoding the β3a subunit of a human calcium sensitive potassium channel (SEQ. ID. NO.:3). The start ATG codon is at position 341-343; the stop codon is at position 1172-1174. **FIG. 2B** shows the deduced amino acid sequence (SEQ. ID. NO.:4) of the β3a subunit.

[0015] **FIG. 3A** shows a DNA sequence encoding the β3b subunit of a human calcium sensitive potassium channel (SEQ. ID. NO.:5). The start ATG codon is at position 796-798; the stop codon is at position 1567-1569. **FIG. 3B** shows the deduced amino acid sequence (SEQ. ID. NO.:6) of the β3b subunit.

[0016] **FIG. 4A** shows a DNA sequence encoding the β3c subunit of a human calcium sensitive potassium channel (SEQ. ID. NO.:7). The start ATG codon is at position 869-871; the stop codon is at position 1694-1696. **FIG. 4B** shows the deduced amino acid sequence (SEQ. ID. NO.:8) of the β3c subunit.

[0017] **FIG. 5A** shows a DNA sequence encoding the β3d subunit of a human and calcium sensitive potassium channel (SEQ. ID. NO.:9). The start ATG codon is at position 457-459; the stop codon is at position 1294-1296. **FIG. 5B** shows the deduced amino acid sequence (SEQ. ID. NO.:10) of the β3d subunit.

[0018] **FIG. 6** shows an alignment of the deduced amino acid sequences of the human calcium sensitive potassium channel β1 (SEQ. ID. NO.:11), β2 (SEQ. ID. NO.:2), β3a (SEQ. ID. NO.:4), β3b (SEQ. ID. NO.:6), β3c (SEQ. ID. NO.:8), and β3d (SEQ. ID. NO.:10) subunits.

[0019] **FIG. 7** shows the effect of the co-expression of the novel β subunits of the present invention on the electrophysiological properties of the ion channel formed by the α subunit of a human calcium sensitive potassium channel. **FIG. 7A** shows the current-voltage relations recorded in inside-out patches expressing calcium sensitive potassium channel α or α and β subunits. α and β subunit cRNAs were co-injected in 1:10 molar ratio (β in excess) to detect maximum effects. The voltage clamp protocol consisted of a pre-pulse to −160 mV (200 ms), followed by 20 mV depolarizing steps from −80 to +80 mV (500 ms); holding potential was −80 mV; internal Ca^{2+} was 30 μM. Subunits β3b and β3d did not induce noticeable changes in the kinetics and voltage dependence of the channels formed by α subunits, although they might decrease current density. **FIG. 7B**: Boltzmann equations were fit to normalized conductances for the records shown in **7A**, which were calculated from peak currents and plotted as function of test potential. $V_{1/2}$ values are: 20 mV (α subunit alone); −55 mV (α+β2 subunit); 45.36 mV (α+β3a subunit); 20 mV (α+β3c subunit). **FIG. 7C** shows that co-expression of β3 subunit RNAs in molar excess of α subunit RNAs (up to 10×) reduced, but did not eliminate, a non-inactivating component of calcium sensitive potassium channel current. Inactivation rates and fractional inactivating current were calculated as described in Example 2.

[0020] **FIG. 8A**-N shows the genomic sequence of GenBank accession number AC007823.4 (SEQ. ID. NO.:20). The different splice variants of the β3 subunits are contained in nucleotides 1-40,467. The β3a-specific sequence is at positions 17,404-17,806; the β3b-specific sequence is at positions 24,710-25,507; the β3c/d sequence is at positions 32,590-33,514; the beginning of the β3 core sequence is at positions 33,515-33,705. The sequences involved in tissue specific expression (e.g., promoters, enhancers, repressors) are likely to be located in nucleotides 1-17,404.

DETAILED DESCRIPTION OF THE INVENTION

[0021] For the purposes of this invention:

[0022] "Substantially free from other proteins" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Whether a given human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein preparation is substantially free from other proteins can be determined by conventional techniques of assessing protein purity such as, e.g., sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, e.g., silver staining or immunoblotting.

[0023] "Substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit nucleic acids. Whether a given human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d

subunit DNA preparation is substantially free from other nucleic acids can be determined by conventional techniques of assessing nucleic acid purity such as, e.g., agarose gel electrophoresis combined with appropriate staining methods, e.g., ethidium bromide staining, Northern or Southern blotting, or by sequencing.

[0024] A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (e.g., arginine for lysine; glutamic acid for aspartic acid); substitution of one aromatic amino acid (tryptophan, tyrosine, or phenylalanine) for another.

[0025] A polypeptide has "substantially the same biological activity as human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit" if that polypeptide is able to combine with a human calcium sensitive potassium channel α subunit thereby forming a functional potassium channel where the polypeptide confers upon the α subunit properties similar to those conferred by the β2, β3a, β3b, β3c, or β3d subunits and where the polypeptide has an amino acid sequence that is at least about 50% identical to SEQ. ID. NO.:2, 4, 6, 8, or 10 when measured by such standard programs as BLAST or FASTA. For example, a polypeptide that is 50% identical in amino acid sequence to β3a (SEQ. ID. NO.:4) and is able to confer upon the α subunit properties such that electrophysiological measurements of the ion channel formed by the polypeptide and the α subunit result in graphs such as those shown in **FIG. 7A**-C for the β3a subunit and the α subunit is a polypeptide that has "substantially the same biological activity as human calcium sensitive potassium channel β3a subunit."

[0026] The present invention relates to the identification and cloning of DNAs encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits, components of human calcium sensitive potassium channels. Expressed sequence tags (ESTs) (GenBank accession numbers AA904191, AI299145 and AI301175) were identified by searching databases for sequences with homology to the β1 subunit. The cDNAs encoding the ESTs were purchased and sequenced in both directions. The clone encoding AA904191 was determined to encode the entire β2 subunit, since it contained in frame stop codons 5' to the start ATG of the open reading frame and the entire open reading frame.

[0027] The β2 coding sequence was then used to search the databases for additional β subunits. Contigs were assembled from the identified ESTs and used to search the database once again. Several ESTs were identified in this iterative manner (GenBank accession numbers AA195381, AA236930, AA236968, AA279911, AA761761 and AA934876). Available cDNAs encoding these ESTs were purchased and sequenced in both directions. None of these clones were full length. Because most were isolated in a preparation of tonsils enriched for B-cells, we performed 5' RACE (rapid amplification of cDNA ends) using gene-specific oligonucleotides in the 3' untranslated region (UTR) and commercially prepared cDNA from human spleen, another tissue rich in B-cells (Clontech catalog # 7412-1), as the template. Multiple DNA fragments were amplified in this manner, cloned and sequenced in both directions. Sequencing revealed 4 subfamilies of full length clones, differing only in their 5' ends: β3a, β3b, β3c, and β3d.

[0028] The human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunits of the present invention exhibit tissue specific patterns of expression. Northern blotting of mRNAs isolated from various tissues has shown that the β2 subunit is expressed predominately in uterus, heart, ovary, thyroid, fetal kidney, adrenal medulla, and pancreas; the β3a subunit is expressed predominately in heart and skeletal muscle; the β3b subunit is expressed in most tissues examined except for brain, skeletal muscle and testes. The β3c and/or β3d subunits have been found in pancreas.

[0029] The tissue specific expression patterns of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunits support the hypothesis that these different subunits may contribute to the functional diversity of calcium sensitive potassium channels observed in different tissues. Activators and inhibitors of specific calcium sensitive potassium channels containing specific subunits may, therefore, have pharmacological efficacy in different pathological conditions, depending on the subunit composition of the calcium sensitive potassium channels involved in the specific pathological condition.

[0030] Chromosomal mapping studies have shown that both the β2 and β3 subunits map to human chromosome 3q23-ter. The β subunits of the present invention have about 30-45% amino acid sequence identity to the previously known human β1 subunit (GenBank accession no. U25138). The β2 and β3 subunits of the present invention have about 40% amino acid sequence identity to each other. The β3a, β3b, β3c, or β3d subunits differ only in their extreme N-terminal 1-20 amino acids, and are alternatively spliced variants of a single gene. Indeed, a genomic fragment of human DNA has been identified in the GenBank database that contains the 5' domains of β3a, β3b, β3c/d, and the beginning of the conserved core in a contiguous fragment (accession number AC007823.4). See **FIG. 8**. Additionally, two bacterial artificial chromosomes (BACs) have been isolated which contain the conserved core domain. One of these BACs also contains β3c/d specific sequence. Therefore, we have identified overlapping BAC clones that together encode the entire β3 open reading frame. The β2 subunit is encoded by a separate gene.

[0031] The present invention provides DNAs encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits that are substantially free from other nucleic acids. The present invention also provides isolated and/or recombinant DNA molecules encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits. The present invention provides DNA molecules substantially free from other nucleic acids comprising the nucleotide sequences shown in SEQ. ID. NOs.:1, 3, 5, 7, or 9. cDNAs encoding each β3 subunit have been isolated exhibiting a sequence polymorphism, encoding either a serine or an asparagine at the amino acid position that is equivalent to position 143 of β3b. This represents amino acid 142 of the conserved core domain.

[0032] Accordingly, the present invention includes DNA substantially free from other nucleic acids as well as isolated and/or recombinant DNA encoding a polypeptide selected

from the group consisting of: SEQ. ID. NO.:4; SEQ. ID. NO.:4 with an asparagine at position 163 instead of a serine; SEQ. ID. NO.:6; SEQ. ID. NO.:6 with a serine at position 143 instead of an asparagine; SEQ. ID. NO.:8; SEQ. ID. NO.:8 with an asparagine at position 161 instead of a serine; SEQ. ID. NO.: 10; and SEQ. ID. NO.:10 with a serine at position 165 instead of an asparagine.

[0033] The present invention includes DNA substantially free from other nucleic acids as well as isolated and/or recombinant DNA encoding a polypeptide comprising the conserved β3 core amino acid sequence, positions 2-246 of SEQ. ID. NO.:6.

[0034] The present invention includes isolated DNA molecules as well as DNA molecules that are substantially free from other nucleic acids comprising the coding regions of SEQ. ID. NOs.:1, 3, 5, 7, and 9. Accordingly, the present invention includes isolated DNA molecules and DNA molecules substantially free from other nucleic acids having a sequence comprising positions 271 to 975 of SEQ. ID. NO.:1, positions 341 to 1171 of SEQ. ID. NO.:3, positions 796 to 1566 of SEQ. ID. NO.:5, positions 869 to 1693 of SEQ. ID. NO.:7, or positions 457 to 1293 of SEQ. ID. NO.:9.

[0035] Also included are recombinant DNA molecules having a nucleotide sequence comprising positions 271-975 of SEQ. ID. NO.:1, positions 341 to 1171 of SEQ. ID. NO.:3, positions 796 to 1566 of SEQ. ID. NO.:5, positions 869 to 1693 of SEQ. ID. NO.:7, or positions 457 to 1293 of SEQ. ID. NO.:9. The novel DNA sequences of the present invention encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits, in whole or in part, can be linked with other DNA sequences, i.e., DNA sequences to which human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits are not naturally linked, to form "recombinant DNA molecules" encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits. Such other sequences can include DNA sequences that control transcription or translation such as, e.g., translation initiation sequences, internal ribosome entry sites, promoters for RNA polymerase II, transcription or translation termination sequences, enhancer sequences, sequences that control replication in microorganisms, sequences that confer antibiotic resistance, or sequences that encode a polypeptide "tag" such as, e.g., a polyhistidine tract, the FLAG epitope, the myc epitope, GST, or maltose binding protein. The novel DNA sequences of the present invention can be inserted into vectors such as plasmids, cosmids, viral vectors, P1 artificial chromosomes, or yeast artificial chromosomes.

[0036] The present invention also includes DNA substantially free from other nucleic acids as well as isolated and/or recombinant DNA comprising genomic sequences of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits. The present invention includes DNA substantially free from other nucleic acids as well as isolated and/or recombinant DNA comprising SEQ. ID. NO.:20; positions 1-40,467 of SEQ. ID. NO.:20; positions 17,404-17,806 of SEQ. ID. NO.:20; positions 24,710-25,507 of SEQ. ID. NO.:20; positions 32,590-33,514 of SEQ. ID. NO.:20; positions 33,515-33,705 of SEQ. ID. NO.:20; or positions 1-17,404 of SEQ. ID. NO.:20.

[0037] Included in the present invention are DNA sequences that hybridize to at least one of SEQ. ID. NOs:1, 3, 5, 7, 9, or 20 under conditions of high stringency. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65° C. in buffer composed of 5×SSC, 10× Denhardt's solution, 50% Formamide, 2% SDS and 100 μg/ml denatured salmon sperm DNA. Hybridization of 32P-labelled, random primed probe is carried out in 5×SSPE, 10× Denhardts solution, 50% Formamide, 2% SDS, 100 ug/ml salmon sperm DNA at 42° C. overnight. Washing of filters is done in 2×SSC, 0.05% SDS at 42° C. for 40 minutes, followed by 0.1×SSC, 0.05% SDS at 65° C. for 40 minutes.

[0038] Other procedures using conditions of high stringency would include either a hybridization carried out in 5×SSC, 5× Denhardt's solution, 50% formamide at 42° C. for 12 to 48 hours or a washing step carried out in 0.2×SSPE, 0.2% SDS at 65° C. for 30 to 60 minutes.

[0039] Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, e.g., Sambrook, Fritsch, and Maniatis, 1989, *Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.*

[0040] The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequences of SEQ. ID. NOs:1, 3, 5, 7, or 9 but still encodes the same human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins as SEQ. ID. NOs:2, 4, 6, 8, or 10. Such synthetic DNAs are intended to be within the scope of the present invention.

[0041] Mutated forms of SEQ. ID. NOs:1, 3, 5, 7, or 9 are intended to be within the scope of the present invention. In particular, mutated forms of SEQ. ID. NOs:1, 3, 5, 7, or 9 which encode proteins that either do not interact with an α subunit or which when combined with α subunits give rise to calcium sensitive potassium channels having altered voltage dependence, calcium sensitivity, current kinetics (such as activation, inactivation or deactivation), or pharmacologic properties as compared to wild-type calcium sensitive potassium channels are within the scope of the present invention. Such mutant forms can differ from SEQ. ID. NOs:1, 3, 5, 7, or 9 by having nucleotide deletions, substitutions, or additions.

[0042] Also intended to be within the scope of the present invention are RNA molecules having sequences corresponding to SEQ. ID. NOs:1, 3, 5, 7, or 9. Antisense nucleotides, DNA or RNA, that are the reverse complements of SEQ. ID. NOs:1, 3, 5, 7, or 9, or portions thereof, are also within the scope of the present invention. In addition, polynucleotides based on SEQ. ID. NOs:1, 3, 5, 7, or 9 in which a small number of positions are substituted with non-natural or modified nucleotides such as inosine, methyl-cytosine, or deaza-guanosine are intended to be within the scope of the present invention. Polynucleotides of the present invention can also include sequences based on SEQ. ID. NOs:1, 3, 5,

7, or 9 but in which non-natural linkages between the nucleotides are present. Such non-natural linkages can be, e.g., methylphosphonates, phosphorothioates, phosphorodithionates, phosphoroamidites, and phosphate esters. Polynucleotides of the present invention can also include sequences based on SEQ. ID. NOs:1, 3, 5, 7, or 9 but having de-phospho linkages as bridges between nucleotides, e.g., siloxane, carbonate, carboxymethyl ester, acetamidate, carbamate, and thioether bridges. Other internucleotide linkages that can be present include N-vinyl, methacryloxyethyl, methacrylamide, or ethyleneimine linkages. Peptide nucleic acids based upon SEQ. ID. NOs:1, 3, 5, 7, or 9 are also included in the present invention.

[0043] Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Such recombinant host cells can be cultured under suitable conditions to produce human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Such recombinant host cells are also useful in the methods of identifying activators and inhibitors of calcium sensitive potassium channels described herein. An expression vector containing DNA encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be used for the expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, amphibian cells such as *Xenopus* oocytes, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cells and cell lines which are suitable for recombinant expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and which are widely available, include but are not limited to, L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), CPAE (ATCC CCL 209), Saos-2 (ATCC HTB-85), ARPE-19 human retinal pigment epithelium (ATCC CRL-2302), *Xenopus* melanophores, and *Xenopus* oocytes.

[0044] A variety of mammalian expression vectors can be used to express recombinant human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pMC1neo (Stratagene), pSG5 (Stratagene), pcDNAI and pcDNAramp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pIZD35 (ATCC 37565), and pSV2-dhfr (ATCC 37146). Another suitable vector is the PT7TS oocyte expression vector.

[0045] Following expression in recombinant cells, human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be purified by conventional techniques to a level that is substantially free from other proteins. Techniques that can be used include ammonium sulfate precipitation, hydrophobic or hydrophilic interaction chromatography, ion exchange chromatography, affinity chromatography, phosphocellulose chromatography, size exclusion chromatography, preparative gel electrophoresis, and alcohol precipitation. In some cases, it may be advantageous to employ protein denaturing and/or refolding steps in addition to such techniques.

[0046] Certain voltage-gated potassium channel subunits have been found to require the expression of other voltage-gated potassium channel subunits in order to be properly expressed at high levels and inserted in membranes. For example, co-expression of KCNQ3 appears to enhance the expression of KCNQ2 in *Xenopus* oocytes (Wang et al., 1998, Science 282:1890-1893). Also, some voltage-gated potassium channel α subunits require other related αsubunits (Jegla and Salkoff, 1997, J. Neurosci. 17:32-44) or Kvβ2 subunits (Shi et al., 1995, Neuron 16:843-852). Accordingly, the recombinant expression of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins may, under certain circumstances, benefit from the co-expression of other proteins and such co-expression is intended to be within the scope of the present invention. A particularly preferred form of co-expression is the co-expression of a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein (or combinations thereof) with a human calcium sensitive potassium channel α subunit protein. Such co-expression can be effected by transfecting an expression vector encoding a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein into a cell that naturally expresses a human calcium sensitive potassium channel α subunit protein. Alternatively, an expression vector encoding a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein can be transfected into a cell in which an expression vector encoding a human calcium sensitive potassium channel α subunit protein has also been transfected. Preferably, such a cell does not naturally express human calcium sensitive potassium channel α or β subunits.

[0047] The present invention includes human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunit proteins substantially free from other proteins. The deduced amino acid sequences of the full-length human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunit proteins are shown in SEQ. ID. NOs.:2, 4, 6, 8, and 10, respectively. Thus, the present invention includes human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunit proteins substantially free from other proteins having the amino acid sequences SEQ. ID. NO.:2, SEQ. ID. NO.:4; SEQ. ID. NO.:4 with an asparagine at position 163 instead of a serine; SEQ. ID. NO.:6; SEQ. ID. NO.:6 with a serine at position 143 instead of an asparagine; SEQ. ID. NO.:8; SEQ. ID. NO.:8 with an asparagine at position 161 instead of a serine; SEQ. ID. NO.:10; and SEQ. ID. NO.:10 with a serine at position 165 instead of an asparagine. The present invention also includes isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunit proteins having the amino acid sequences SEQ. ID. NO.:2, SEQ. ID. NO.:4; SEQ. ID. NO.:4 with an asparagine at position 163 instead of a serine; SEQ. ID. NO.:6; SEQ. ID. NO.:6 with a serine at position 143 instead of an asparagine; SEQ. ID. NO.:8; SEQ. ID.

NO.:8 with an asparagine at position 161 instead of a serine; SEQ. ID. NO.: 10; and SEQ. ID. NO.:10 with a serine at position 165 instead of an asparagine.

[0048] Mutated forms of human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunit proteins are intended to be within the scope of the present invention. In particular, mutated forms of SEQ. ID. NOs:2, 4, 6, 8, and 10 that give rise to calcium sensitive potassium channels having altered electrophysiological or pharmacological properties when combined with α subunits are within the scope of the present invention.

[0049] As with many proteins, it is possible to modify many of the amino acids of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and still retain substantially the same biological activity as for the original proteins. Thus, the present invention includes modified human calcium sensitive potassium channel β2, β3a, β3b, β3c, and β3d subunit proteins which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as naturally occurring human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, e.g., *Molecular Biology of the Gene*, Watson et al., 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ. ID. NOs:2, 4, 6, 8, or 10 wherein the polypeptides still retain substantially the same biological activity as naturally occurring human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ. ID. NOs:2, 4, 6, 8, or 10 wherein the polypeptides still retain substantially the same biological activity as naturally occurring human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in conserved positions. Conserved positions are those positions in which the human calcium sensitive potassium channel β1, β2, and any of the β3 subunits all have the same amino acid (see **FIG. 6**).

[0050] The human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins of the present invention may contain post-translational modifications, e.g., covalently linked carbohydrate, phosphorylation, myristoylation, palmyltoylation, etc.

[0051] The present invention also includes chimeric human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Chimeric human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins consist of a contiguous polypeptide sequence of at least a portion of a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein fused to a polypeptide sequence that is not from a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein.

[0052] The present invention also includes isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and DNA encoding these isolated subunits. Use of the term "isolated" indicates that the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein or DNA has been removed from its normal cellular environment. Thus, an isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein is the only protein present, but instead means that the isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein is at least 95% free of non-amino acid material (e.g., nucleic acids, lipids, carbohydrates) naturally associated with the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein. Thus, a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein that is expressed in bacteria or even in eukaryotic cells which do not naturally (i.e., without human intervention) express it through recombinant means is an "isolated human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein."

[0053] It is known that certain potassium channels subunits can interact to form heteromeric structures resulting in functional potassium channels. For example, KCNQ2 and KCNQ3 can assemble to form a heteromeric functional potassium channel (Wang et al., 1998, Science 282:1890-1893). Accordingly, it is believed likely that the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins of the present invention will also be able to form heteromeric structures with other proteins where such heteromeric structures constitute functional potassium channels. Thus, the present invention includes such heteromers comprising human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Preferred heteromers are those in which the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins of the present invention forms heteromers with calcium sensitive potassium channel α subunits.

[0054] DNA encoding the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be obtained by methods well known in the art. For example, a cDNA fragment encoding full-length human calcium sensitive potassium channel β2 subunit protein can be isolated from human uterus, ovary or pancreas cDNA by using the polymerase chain reaction (PCR) employing suitable primer pairs. Such primer pairs can be selected based upon the DNA sequence encoding the human calcium sensitive potassium channel β2 subunit protein shown in **FIG. 1A** as SEQ. ID. NO.:1. Suitable primer pairs would be, e.g.:

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5'-AAG ATG TTT ATA TGG ACC AGT  (SEQ. ID. NO.: 12)
GGC-3'
and

5'-ACT CAT AAC AGA CTG CAC GTT  (SEQ. ID. NO.: 13)
AC-3'.
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[0055] The above and subsequent primers are meant to be illustrative only; one skilled in the art would readily be able to design other suitable primers based upon SEQ. ID. NO.:1. Such primers could be produced by methods of oligonucleotide synthesis that are well known in the art.

[0056] In a similar manner, PCR primers can be selected and produced for the other human calcium sensitive potassium channel subunit proteins of the present invention. For example, for the human calcium sensitive potassium channel β3a subunit, suitable primer pairs would be, e.g.:

5'-GTC ATG CAG CCC TTC AGC ATC (SEQ. ID. NO.: 14)
CC-3'
and

5'-TTG CAG AAA TCA CAG ACA TCT (SEQ. ID. NO.: 15)
GAA-3'.

[0057] A suitable cDNA template from which the human calcium sensitive potassium channel β3a subunit can be isolated is human heart, skeletal muscle or spleen cDNA.

[0058] For the human calcium sensitive potassium channel β3b subunit, suitable primer pairs would be, e.g.:

5'-GCA ATG ACA GCC TTT CCT GCC (SEQ. ID. NO.: 16)
TC-3'
and

5'-TTG CAG AAA TCA CAG ACA TCT (SEQ. ID. NO.: 15)
GAA-3'.

[0059] A suitable cDNA template from which the human calcium sensitive potassium channel β3b subunit can be isolated is human spleen cDNA.

[0060] For the human calcium sensitive potassium channel β3c subunit, suitable primer pairs would be, e.g.:

5'-GAA ATG TTC CCC CTT CTT TAT (SEQ. ID. NO.: 17)
GAG-3'
and

5'-TTG CAG AAA TCA CAG ACA TCT (SEQ. ID. NO.: 15)
GAA-3'.

[0061] A suitable cDNA template from which the human calcium sensitive potassium channel β3c subunit can be isolated is human pancreas or spleen cDNA.

[0062] For the human calcium sensitive potassium channel β3d subunit, suitable primer pairs would be, e.g.:

5'-GAG ATG GAC TTT TCA CCA AGC (SEQ. ID. NO.: 18)
TCT-3'
and

5'-TTG CAG AAA TCA CAG ACA TCT (SEQ. ID. NO.: 15)
GAA-3'.

[0063] A suitable cDNA template from which the human calcium sensitive potassium channel β3d subunit can be isolated is human pancreas or spleen cDNA.

[0064] PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM $MgCl_2$, 200 μM for each dNTP, 50 mM KCl, 0.2 μM for each primer, 10 ng of DNA template, 0.05 units/μl of AmpliTaq. The reactions are heated at 95° C. for 3 minutes and then cycled 25 times using the cycling parameters of 95° C., 20 seconds, 62° C., 20 seconds, 72° C., 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in *PCR Primer, A Laboratory Manual*, edited by C. W. Dieffenbach and G. S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or *PCR Protocols: A Guide to Methods and Applications*, Michael et al., eds., 1990, Academic Press.

[0065] Since the calcium sensitive potassium channel subunits of the present invention are highly homologous to one another, and to other potassium channel subunits, it is desirable to sequence the clones obtained by the herein-described methods, in order to verify that the desired calcium sensitive potassium channel β subunits have in fact been obtained.

[0066] By these methods, cDNA clones encoding the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be obtained. These cDNA clones can be cloned into suitable cloning vectors or expression vectors, e.g., the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, Calif.). Human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can then be produced by transfecting expression vectors encoding the subunits or portions thereof into suitable host cells and growing the host cells under appropriate conditions. Human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can then be isolated by methods well known in the art.

[0067] As an alternative to the above-described PCR methods, cDNA clones encoding the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be isolated from cDNA libraries using as a probe oligonucleotides specific for each human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit and methods well known in the art for screening cDNA libraries with oligonucleotide probes. Such methods are described in, e.g., Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.; Glover, D. M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K., Vol. I, II. Oligonucleotides that are specific for particular human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits and that can be used to screen cDNA libraries can be readily designed based upon the DNA sequences shown in **FIGS. 1-5** and can be synthesized by methods well-known in the art.

[0068] Genomic clones containing the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes can be obtained from commercially available human PAC, YAC, or BAC libraries available from Research Genetics, Huntsville, Ala. Alternatively, one may prepare genomic libraries, e.g., in P1 artificial chromosome vectors, from which genomic clones containing the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes can be isolated, using probes based upon the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit DNA sequences disclosed herein. Methods of preparing such libraries are known in the art (see, e.g., Ioannou et al., 1994, Nature Genet. 6:84-89).

[0069] The novel DNA sequences of the present invention can be used in various diagnostic methods. The present invention provides diagnostic methods for determining whether a patient carries a mutation in one or more of the

human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes. In broad terms, such methods comprise determining the DNA sequence of a region in or near one or more of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes from the patient and comparing that sequence to the sequence from the corresponding region of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes from a non-affected person, i.e., a person who does not have the condition which is being diagnosed, where a difference in sequence between the DNA sequence of the gene from the patient and the DNA sequence of the gene from the non-affected person indicates that the patient has a mutation in one or more of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes.

[0070] The present invention also provides oligonucleotide probes, based upon the sequences of SEQ. ID. NOs:1, 3, 5, 7, 9, or 20 that can be used in diagnostic methods to identify patients having mutated forms of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits, to determine the level of expression of RNA encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits, or to isolate genes homologous to human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits. In particular, the present invention includes DNA oligonucleotides comprising at least about 10, 15, or 18 contiguous nucleotides of a sequence selected from the group consisting of: SEQ. ID. NOs:1, 3, 5, 7, 9, and 20 where the oligonucleotide probe comprises no stretch of contiguous nucleotides longer than 5 of a sequence selected from the group consisting of: SEQ. ID. NOs:1, 3, 5, 7, 9, and 20 other than the said at least about 10, 15, or 18 contiguous nucleotides. The oligonucleotides can be substantially free from other nucleic acids. Also provided by the present invention are corresponding RNA oligonucleotides. The DNA or RNA oligonucleotide can be packaged in kits for use as probes.

[0071] The present invention makes possible the recombinant expression of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in various cell types.

[0072] The β2, β3a, β3b, β3c, and β3d subunits of the human calcium sensitive potassium channel have been expressed in *Xenopus* oocytes, both by themselves and in combination with an α subunit of a large-conductance calcium-sensitive potassium channel (maxi-K channel). The β subunits do not express currents on their own. However, when co-expressed with the α subunit, the β2, β3a, and β3c subunits induce inactivation of calcium sensitive potassium currents (**FIG. 7**). The rates of inactivation produced by β2, β3a and β3c are dependent upon voltage and internal calcium concentration; inactivation time constants reach a maximum at high depolarizations and high micromolar calcium for β2, β3a and β3c, τ_{inact}~30-40 ms at 80 mV with 30 μM intracellular Ca^{2+}. Measurements of current-voltage dependence obtained in the presence of micromolar intracellular Ca^{2+} demonstrate that β2 subunits induce a large shift in the voltage dependence of activation (~80 mV towards negative potentials, with 30 μM Ca^{2+} in the bath; **FIG. 7B**). This modulatory effect is similar to the one previously described for β1 subunits, which do not induce inactivation. (McManus et al., 1995, Neuron 14:645-650). In contrast, β3a, β3b, β3c, and β3d subunits do not shift the voltage dependence when compared to channels containing only α subunits (**FIG. 7B**).

[0073] The present invention also makes possible the development of assays that measure the biological activity of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Such assays using recombinantly expressed human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are especially of interest. Such assays can be used to screen libraries of compounds or other sources of compounds to identify compounds that are activators or inhibitors of the activity of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Such identified compounds can serve as "leads" for the development of pharmaceuticals that can be used to treat patients having diseases in which it is beneficial to enhance or suppress calcium sensitive potassium channel activity.

[0074] In versions of the above-described assays, calcium sensitive potassium channels containing mutant human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are used and inhibitors or activators of the activity of the mutant calcium sensitive potassium channels are identified.

[0075] Preferred cell lines for recombinant expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are those which do not express endogenous potassium channels (e.g., CV-1, NIH-3T3, CHO-K1, COS-7). Such cell lines can be loaded with ^{86}Rb, an ion which can pass through potassium channels. The ^{86}Rb-loaded cells can be exposed to collections of substances (e.g., combinatorial libraries, natural products, analogues of lead compounds produced by medicinal chemistry) and those substances that are able to alter ^{86}Rb efflux identified. Such substances are likely to be activators or inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

[0076] Activators and inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are likely to be substances that are capable of binding to calcium sensitive potassium channels. Accordingly, one type of assay determines whether one or more of a collection of substances is capable of such binding.

[0077] Accordingly, the present invention provides a method for identifying substances that bind to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

[0078] (a) providing cells expressing a calcium sensitive potassium channel containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

[0079] (b) exposing the cells containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins to a substance that is not known to bind calcium sensitive potassium channels;

[0080] (c) determining the amount of binding of the substance to the cells;

[0081] (d) comparing the amount of binding in step (c) to the amount of binding of the substance to control cells where the control cells are substantially identical to the cells of step (a) except that the control cells do not express human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

[0082] where if the amount of binding in step (c) is greater than the amount of binding of the substance to control cells, then the substance binds to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

[0083] Another version of this assay makes use of compounds that are known to bind to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. New binders are identified by virtue of their ability to potentiate, prevent, or displace the binding of the known compounds. Substances that have this ability are likely themselves to be inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

[0084] Accordingly, the present invention includes a method of identifying substances that bind calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and thus are likely to be inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

[0085] (a) providing cells expressing calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

[0086] (b) exposing the cells to a compound that is known to bind to the calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

[0087] (c) determining the amount of binding of the compound to the cells in the presence and in the absence of a substance not known to bind to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

[0088] where if the amount of binding of the compound in the presence of the substance differs from that in the absence of the substance, then the substance binds calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and is likely to be an inhibitor or activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

[0089] Generally, the known compound is labeled (e.g., radioactively, enzymatically, fluorescently) in order to facilitate measuring its binding to the calcium sensitive potassium channels.

[0090] Once a substance has been identified by the above-described methods, it can be assayed in functional tests, such as those described herein, in order to determine whether it is an inhibitor or an activator.

[0091] In particular embodiments, the compound known to bind calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins is selected from the group consisting of: charybdotoxin, iberiotoxin, and dehydrosoyasaponin.

[0092] The present invention includes a method of identifying activators or inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

[0093] (a) recombinantly expressing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins or mutant human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in a host cell so that the recombinantly expressed human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins form calcium sensitive potassium channels by forming heteromers with other calcium sensitive potassium channel subunit proteins;

[0094] (b) measuring the biological activity of the calcium sensitive potassium channels formed in step (a) in the presence and in the absence of a substance suspected of being an activator or an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

[0095] where a change in the biological activity of the calcium sensitive potassium channels formed in step (a) in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

[0096] It may be advantageous to recombinantly express other subunits of calcium sensitive potassium channels such as, e.g., an α subunit. Alternatively, it may be advantageous to use host cells that endogenously express such other subunits.

[0097] In particular embodiments, the biological activity is the production of a calcium sensitive potassium current, a FRET signal, or the efflux of ^{86}Rb.

[0098] In particular embodiments, a vector encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins is transferred into *Xenopus* oocytes in order to cause the expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in the oocytes. Alternatively, RNA encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be prepared in vitro and

injected into the oocytes, also resulting in the expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in the oocytes. Following expression of the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in the oocytes, and following the formation of calcium sensitive potassium channels containing these subunits and other calcium sensitive potassium channel subunits (which other subunits may also be transferred into the oocytes), membrane currents are measured after the transmembrane voltage and/or internal calcium concentration is changed in steps. A change in membrane current is observed when the calcium sensitive potassium channels open or close, allowing or inhibiting potassium ion flow, respectively. Similar oocyte studies were reported for KCNQ2 and KCNQ3 potassium channels in Wang et al., 1998, Science 282:1890-1893 and this reference and references cited therein can be consulted for guidance as to how to carry out such studies.

[0099] Inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be identified by exposing the oocytes to collections of substances and determining whether the substances can block or diminish, or activate or enhance the membrane currents observed in the absence of the substance.

[0100] Accordingly, the present invention provides a method of identifying inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

[0101] (a) expressing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in a heterologous system such that calcium sensitive potassium channels containing the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are formed;

[0102] (b) changing the transmembrane potential or internal calcium concentration of the heterologous system in the presence and the absence of a substance suspected of being an inhibitor or activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

[0103] (c) measuring membrane potassium currents following step (b);

[0104] where if the membrane potassium currents measured in step (c) are greater in the absence rather than in the presence of the substance, then the substance is an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

[0105] where if the membrane potassium currents measured in step (c) are less in the absence rather than in the presence of the substance, then the substance is an activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

[0106] In particular embodiments, the heterologous system is selected from the group consisting of: *Xenopus* oocytes and a mammalian cell line.

[0107] The present invention also includes assays for the identification of activators and inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins that are based upon fluorescence resonance energy transfer (FRET) between a first and a second fluorescent dye where the first dye is bound to one side of the plasma membrane of a cell expressing calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and the second dye is free to shuttle from one face of the membrane to the other face in response to changes in membrane potential. In certain embodiments, the first dye is impenetrable to the plasma membrane of the cells and is bound predominately to the extracellular surface of the plasma membrane. The second dye is trapped within the plasma membrane but is free to diffuse within the membrane. At normal (i.e., negative) resting potentials of the membrane, the second dye is bound predominately to the inner surface of the extracellular face of the plasma membrane, thus placing the second dye in close proximity to the first dye. This close proximity allows for the generation of a large amount of FRET between the two dyes. Following membrane depolarization, the second dye moves from the extracellular face of the membrane to the intracellular face, thus increasing the distance between the dyes. This increased distance results in a decrease in FRET, with a corresponding increase in fluorescent emission derived from the first dye and a corresponding decrease in the fluorescent emission from the second dye. In this way, the amount of FRET between the two dyes can be used to measure the polarization state of the membrane. For a fuller description of this technique, see González & Tsien, 1997, Chemistry & Biology 4:269-277. See also González & Tsien, 1995, Biophys. J. 69:1272-1280 and U.S. Pat. No. 5,661,035.

[0108] In certain embodiments, the first dye is a fluorescent lectin or a fluorescent phospholipid that acts as the fluorescent donor. Examples of such a first dye are: a coumarin-labeled phosphatidylethanolamine (e.g., N-(6-chloro-7-hydroxy-2-oxo-2H-1-benzopyran-3-carboxamidoacetyl)-dimyristoylphosphatidyl-ethanolamine) or N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-dipalmitoylphosphatidylethanolamine); a fluorescently-labeled lectin (e.g., fluorescein-labeled wheat germ agglutinin). In certain embodiments, the second dye is an oxonol that acts as the fluorescent acceptor. Examples of such a second dye are: bis(1,3-dialkyl-2-thiobarbiturate)trimethineoxonols (e.g., bis(1,3-dihexyl-2-thiobarbiturate)trimethineoxonol) or pentamethineoxonol analogues (e.g., bis(1,3-dihexyl-2-thiobarbiturate)pentamethineoxonol; or bis(1,3-dibutyl-2-thiobarbiturate)pentamethineoxonol). See González & Tsien, 1997, Chemistry & Biology 4:269-277 for methods of synthesizing various dyes suitable for use in the present invention. In certain embodiments, the assay may comprise a natural carotenoid, e.g., astaxanthin, in order to reduce photodynamic damage due to singlet oxygen.

[0109] The above described assays can be utilized to discover activators and inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Such assays will generally utilize cells that express calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d

subunit proteins, e.g., by transfection with expression vectors encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and, optionally, other calcium sensitive potassium channel subunits. In such cells, depolarization of the membrane potential as well as increases in intracellular calcium concentration will tend to open the calcium sensitive potassium channels. This will result in potassium efflux, tending to counteract the depolarization. In other words, the cells will tend to repolarize. The presence of an inhibitor of the calcium sensitive potassium channel will prevent, or diminish, this repolarization. Thus, membrane potential will tend to become more positive (i.e., depolarized) in the presence of inhibitors. Activators of the calcium sensitive potassium channel will open this channel and thus tend to hyperpolarize the membrane potential. Changes in membrane potential (depolarizations and hyperpolarizations) that are caused by inhibitors and activators of calcium sensitive potassium channels can be monitored by the assays using FRET described above.

[0110] Accordingly, the present invention provides a method of identifying activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

[0111] (a) providing test cells comprising:

[0112] (1) an expression vector that directs the expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in the cells so that calcium sensitive potassium channels containing human β2, β3a, β3b, β3c, or β3d subunit proteins are formed in the cells;

[0113] (2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane of the cells; and

[0114] (3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane of the cells to the other face in response to changes in membrane potential;

[0115] (b) exposing the test cells to a substance that is suspected of being an activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

[0116] (c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells that have been exposed to the substance;

[0117] (d) comparing the amount of FRET exhibited by the test cells that have been exposed to the substance with the amount of FRET exhibited by control cells;

[0118] wherein if the amount of FRET exhibited by the test cells is greater than the amount of FRET exhibited by the control cells, the substance is an activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d, -subunit proteins;

[0119] where the control cells are either (1) cells that are essentially the same as the test cells except that they do not comprise at least one of the items listed at (a) (1)-(3) but have been exposed to the substance; or (2) test cells that have not been exposed to the substance.

[0120] The present invention also provides a method of identifying inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

[0121] (a) providing test cells comprising:

[0122] (1) an expression vector that directs the expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in the cells so that calcium sensitive potassium channels containing human β2, β3a, β3b, β3c, or β3d subunit proteins are formed in the cells;

[0123] (2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane of the cells; and

[0124] (3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane of the cells to the other face in response to changes in membrane potential;

[0125] (b) exposing the test cells to a substance that is suspected of being an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

[0126] (c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells that have been exposed to the substance;

[0127] (d) comparing the amount of FRET exhibited by the test cells that have been exposed to the substance with the amount of FRET exhibited by control cells;

[0128] wherein if the amount of FRET exhibited by the test cells is less than the amount of FRET exhibited by the control cells, the substance is an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

[0129] where the control cells are either (1) cells that are essentially the same as the test cells except that they do not comprise at least one of the items listed at (a) (1)-(3) but have been exposed to the substance; or (2) test cells that have not been exposed to the substance.

[0130] In a variation of the assay described above, instead of the cell's membrane potential being allowed to reach steady state on its own, the membrane potential is artificially set at a potential in which the calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are open. This can be done, e.g., by variation of the external K^+ concentration in a known manner (e.g., increased concentrations of external K^+). If such cells, having open calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, are exposed to inhibitors, the calcium

sensitive potassium channels will be blocked, and the cells' membrane potentials will be depolarized. This depolarization can be observed as a decrease in FRET.

[0131] In a variation of the assay described above, instead of the cell's membrane potential being allowed to reach steady state on its own, the membrane potential is artificially set at a potential in which the calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins are open by coexpression of another depolarizing current. If such cells, having open calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, are exposed to inhibitors, the calcium sensitive potassium channels will be blocked, and the cells' membrane potentials will be depolarized. This depolarization can be observed as a decrease in FRET. If such cells, having open calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, are exposed to activators, the balance of the calcium sensitive potassium current and the additional depolarizing current will shift (i.e., the calcium sensitive potassium current will make a larger contribution to the total current) and the cell's membrane potential will be hyperpolarized. This polarization may be observed as an increase in FRET.

[0132] Accordingly, the present invention provides a method of identifying inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

[0133] (a) providing cells comprising:

[0134] (1) an expression vector that directs the expression of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in the cells so that calcium sensitive potassium channels containing human β2, β3a, β3b, β3c, or β3d subunit proteins are formed in the cells;

[0135] (2) a first fluorescent dye, where the first dye is bound to one side of the plasma membrane of the cells; and

[0136] (3) a second fluorescent dye, where the second fluorescent dye is free to shuttle from one face of the plasma membrane of the cells to the other face in response to changes in membrane potential;

[0137] (b) adjusting the membrane potential of the cells such that the ion channel formed by the calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins is open;

[0138] (c) measuring the amount of fluorescence resonance energy transfer (FRET) in the test cells;

[0139] (d) repeating step (b) and step (c) while the cells are exposed to a substance that is suspected of being an inhibitor or activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

[0140] where if the amount of FRET exhibited by the cells that are exposed to the substance is different than the amount of FRET exhibited by the cells that have not been exposed to the substance, then the substance is an inhibitor or activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

[0141] In particular embodiments of the above-described methods, the cells contain an expression vector encoding a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein. In particular embodiments of the above-described methods, the expression vector is transfected into the test cells.

[0142] In particular embodiments of the above-described methods, the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein has an amino acid sequence selected from the group consisting of: SEQ. ID. NO.:2, 4, 6, 8, and 10.

[0143] In particular embodiments of the above-described methods, the first fluorescent dye is selected from the group consisting of: a fluorescent lectin; a fluorescent phospholipid; a coumarin-labeled phosphatidylethanolamine; N-(6-chloro-7-hydroxy-2-oxo-2H-1-benzopyran-3-carboxamidoacetyl)-dimyristoylphosphatidyl-ethanolamine); N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-dipalmitoyl-phosphatidylethanolamine); and fluorescein-labeled wheat germ agglutinin.

[0144] In particular embodiments of the above-described methods, the second fluorescent dye is selected from the group consisting of: an oxonol that acts as the fluorescent acceptor; bis(1,3-dialkyl-2-thiobarbiturate)trimethineoxonols; bis(1,3-dihexyl-2-thiobarbiturate)trimethineoxonol; bis(1,3-dialkyl-2-thiobarbiturate)quatramethineoxonols; bis(1,3-dialkyl-2-thiobarbiturate)pentamethineoxonols; bis(1,3-dihexyl-2-thiobarbiturate)pentamethineoxonol; bis(1,3-dibutyl-2-thiobarbiturate)pentamethineoxonol); and bis(1,3-dialkyl-2-thiobarbiturate)hexamethineoxonols.

[0145] In a particular embodiment of the above-described methods, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK$^-$) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171), *Xenopus* melanophores, or *Xenopus* oocytes.

[0146] In particular embodiments of the above-described methods, the control cells do not comprise item (a)(1) but do comprise items (a)(2) and (a)(3).

[0147] In assays to identify activators or inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, it may be advantageous to co-express another calcium sensitive potassium channel subunit besides the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit. In particular, it may be advantageous to co-express a calcium sensitive potassium channel α subunit. Preferably, this is done by co-transfecting into the cells an expression vector encoding the other subunit. Suitable other subunits are, e.g., the human calcium sensitive

potassium channel α subunit h-slo (GenBank accession no. U11058), the mouse calcium sensitive potassium channel α subunit m-slo (GenBank accession no. U09383), the small conductance calcium sensitive potassium α subunits (GenBank accession nos. U69883, U69882, AF031815), or the intermediate conductance calcium sensitive potassium channel α subunit (GenBank accession no. AF022797).

[0148] Small regions of genomic sequences in proximity to a gene often regulate the transcription of that gene. These sequences are referred to as cis-acting elements. The proteins that bind these DNA sequences and directly affect the ability of the transcriptional machinery to bind or transcribe the gene are referred to as trans-acting elements. The cis-acting transcriptional regulatory elements are most often 5' of the transcription start site, but have been located within and 3' of the transcribed portion of genes as well. Depending on their effects on the rate of transcription, these sequences can be divided into three categories: promoters, enhancers, and repressors. A promoter independently allows transcription of the gene, while an enhancer increases the rate of transcription but is not capable of inducing transcription independently of the promoter. A repressor element inhibits transcription directed by a promoter element. Methods for identifying these elements are well know in the field and are described in Ausubel et al., eds., 1989, *Current Protocols in Molecular Biology*, sections 9.6-9.8, and 12.0-12.11, John Wiley & Sons, New York, N.Y.

[0149] Accordingly, the novel genomic sequences (SEQ. ID. NO.:20, **FIG. 8**) and isolated BAC clones of the present invention make possible methods for identifying 1) DNA sequences required for transcriptional control of gene expression, 2) proteins involved in transcriptional regulation and 3) compounds which modulate the rate of transcription of the β3 gene. Such assays utilize isolated and/or recombinant DNA comprising portions of SEQ. ID. NO.:20, positions 1 to 17,436, inserted into vectors upstream of the open reading frame of a reporter protein.

[0150] Useful reporter proteins are ones that are not expressed in the cells to be assayed (or are easily distinguished from endogenous proteins), have a linear relationship between the abundance of the transcript and the abundance of the reporter protein, and have a large window between the minimum detection level and saturation of detection system. Ideally, the abundance of the reporter protein is quickly measured by an enzymatic reaction, fluorescence detection, immunoassay or other means. Typical reporter proteins include, but are not limited to, the following: Chloramphenicol Acetyltransferase (CAT), firefly luciferase, Beta-Lactamase, Beta-Galactosidase, Secreted Alkaline Phosphatase (SEAP), human Growth Hormone (hGH), Green Fluorescent Protein (GFP) and GFP derivatives. Reporter vectors incorporating these proteins are commercially available, as are similar reporter vectors containing constitutive promoters, enhancers, or both (Clontech).

[0151] The present invention provides a method for identifying nucleotide sequences involved in transcriptional regulation of β3 gene expression. Once a fragment of at least 6 contiguous nucleotides of DNA from SEQ. ID. NO.:20, positions 1-17,436, has been inserted upstream of the reporter cDNA in a promoter-reporter vector, the vector is then transfected into cells that either do or do not endogenously express one or more of the calcium sensitive potassium channel subunits β3a, β3b, β3c or β3d. Promoter-reporter vectors may contain promoters, enhancers, both, or neither. Transfected cells are then assayed for the amount of reporter protein present. Because both transfection efficiency and transcription rate directly affect reporter protein level, it is useful in these assays to determine the transfection efficiency by co-transfecting a second vector (molar ratio 1:1) containing a distinct reporter behind a constitutive promoter, and determining the fraction of transfected cells.

[0152] In versions of the above assay, vectors are constructed with fragments of SEQ. ID. NO.:20 inserted upstream of a reporter cDNA with no other enhancer or promoter elements. These vectors (with and without fragments of SEQ. ID. NO.:20) are transfected into cells that endogenously express β3 subunits. Calcium sensitive potassium channel β3 subunit promoter elements are identified by the ability of these 5' gene fragments to stimulate reporter expression above the levels observed in the parent vector. The minimum required promoter sequence is then identified by successively deleting regions of the identified promoter fragment, and repeating the assay.

[0153] Another version of the assay incorporates fragments of SEQ. ID. NO.:20 inserted upstream of the reporter cDNA in a promoter-reporter vector containing an enhancer element. These vectors (with and without fragments of SEQ. ID. NO.:20) are transfected into cells that endogenously express β3 subunits. Weak calcium sensitive potassium channel β3 subunit promoter elements are identified by the ability of these 5' gene fragments to stimulate reporter expression above the levels observed in the parent vector. The minimum required weak promoter sequence can then be identified by successively deleting regions of the identified weak promoter fragment and repeating the assay

[0154] A different version of the assay incorporates fragments of SEQ. ID. NO.:20 inserted upstream of the reporter cDNA in a promoter-reporter vector with a constitutive promoter upstream. These vectors (with and without fragments of SEQ. ID. NO.:20) are transfected into cells that do not endogenously express β3 subunits. Calcium sensitive potassium channel β3 subunit repressor elements are identified by the ability of these 5' gene fragments to prevent or reduce reporter expression below the levels observed in the parent vector. The minimum required repressor sequence is then identified by successively deleting regions of the identified repressor fragment and repeating the assay.

[0155] In view of the above, the present invention provides a method of identifying DNA sequences in the β3 gene that promote, enhance, or repress gene transcription comprising:

[0156] (a) constructing a promoter-reporter vector such that fragments of the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) precede the coding cDNA sequence of a reporter gene which encodes a reporter protein;

[0157] (b) transfecting the vector into cells and measuring the abundance of the reporter protein encoded by the vector;

[0158] (c) comparing the abundance of the reporter protein in the cells of step (b) to the abundance of the

reporter protein in cells transfected with the vector without fragments of the promoter region of the β3 gene;

[0159] where fragments of the promoter region of the β3 gene which increase the abundance of the reporter protein in the absence of other promoter elements only in cells which endogenously express β3a, β3b, β3c, or β3d subunits are promoter elements; sequences which decrease the abundance of the reporter protein in the presence of an unrelated constitutive promoter element in cells which do not endogenously express β3a, β3b, β3c, or β3d subunits are repressor elements; and sequences which increase the abundance of the reporter protein in the presence of an unrelated constitutive promoter element in cells which endogenously express β3a, β3b, β3c, or β3d subunits are enhancer elements.

[0160] In particular embodiments, the vector contains promoter or enhancer sequence elements which function independently of the fragments of the promoter region of the β3 gene.

[0161] In particular embodiments, the abundance of the reporter protein is normalized with respect to the fraction of transfected cells.

[0162] The binding of nuclear proteins to these sequences can be confirmed by gel-shift assays. A radiolabeled DNA fragment corresponding to the minimal sequence required to affect transcription is incubated with nuclear protein extracts from cells used to identify the regulatory DNA element, or tissues endogenously expressing β3 subunits. If a protein factor binds that sequence, the mobility in a gel will be altered, resulting in an apparent shift in the size of the radiolabeled fragment.

[0163] Transcription factors often are able to recognize more than one specific nucleotide sequence. As such, variations of sequences identified as minimal promoters, enhancers or repressors necessary for transcriptional regulation of the β3 gene in SEQ. ID. NO.:20, positions 1-17,436, which retain the ability to influence transcription as detected in the above described assays are intended to be included in the present invention.

[0164] Minimal promoter, enhancer or repressor DNA fragments thus identified can then be used to identify and/or isolate proteins that influence transcriptional activity of the β3 gene. Several methods are well known in the field, some of which are described in Ausubel et al., eds., 1989, *Current Protocols in Molecular Biology*, sections 12.0-12.11, John Wiley & Sons, New York, N.Y.

[0165] In one method, gel shift assays described above can be performed with cloned or purified known transcription factors, to determine if they are capable of binding sequences involved in transcriptional regulation. Alternatively, super-shift assays can be performed in which an antibody that recognizes a particular transcription factor is added to the transcription factor-DNA complex. If the antibody binds to the transcription factor, which in turn binds the radiolabeled DNA fragment, the mobility of the complex in a gel is further altered, resulting in a super-shift compared to the DNA alone. Using antibodies that recognize a specific transcription factor, or a class of transcription factors, allows identification of the factors involved in β3 gene regulation. Variations of sequences identified as minimal promoters, enhancers or repressors necessary for transcriptional regulation of the β3 gene in SEQ. ID. NO.:20, positions 1-17, 436, which retain the ability to undergo gel shifts or supershifts as described in the above assays are intended to be included in the present invention.

[0166] In view of the above, the present invention provides a method of identifying DNA sequences in the β3 gene that promote, enhance, or repress gene transcription comprising:

[0167] (a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) with nuclear extracts from cells and separating the incubation on a gel;

[0168] where fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene that migrate differently in a gel ('undergo a shift') after incubation with nuclear extracts from cells are DNA sequences which bind nuclear factors which promote, enhance or repress β3 gene expression.

[0169] In particular embodiments, the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the method of claim **18**.

[0170] In particular embodiments, the cells express 3a, β3b, β3c, or β3d subunits.

[0171] In particular embodiments, the cells do not express β3a, β3b, β3c, or β3d subunits.

[0172] The present invention provides a method of identifying nuclear factors involved in β3 gene transcription regulation comprising:

[0173] (a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) with cloned or purified transcription factors and separating the incubation on a gel;

[0174] where factors which bind β3 gene promoter sequence elements will induce a shift in the migration of the radiolabeled DNA fragments, and are involved in β3 gene transcription regulation.

[0175] In particular embodiments, the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim **18** or **21**.

[0176] The present invention provides a method of identifying nuclear factors involved in β3 gene transcription regulation comprising:

[0177] (a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) with nuclear extracts from cells and separating the incubation on a gel;

[0178] (b) adding an antibody that specifically recognizes a single transcription factor or a family of transcription factors to the incubation of step (a), followed by separating the incubation on a gel;

[0179] where a super-shift in mobility of the double stranded DNA in step (b) as compared to step (a) indicates that a transcription factor recognized by the antibody binds the double stranded DNA.

[0180] In another method, the transcription factors that bind SEQ. ID. NO.:20, positions 1-17,436, and regulate transcription can be purified. DNA fragments corresponding to the minimal sequence required to affect transcription are covalently linked to a matrix (typically an agarose bead). This matrix is then incubated with nuclear extracts of cells that contain factors which bind the minimal element. The matrix is then washed free of non-specific proteins and the factor(s) are eluted with an excess of the DNA element, or by denaturation. Purified proteins can then be identified by immunoassay, protein sequencing, or other means.

[0181] Accordingly, the present invention provides a method of identifying nuclear factors involved in β3 gene transcription regulation comprising:

[0182] (a) attaching fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) to a stable matrix;

[0183] (b) incubating nuclear extracts from cells with the matrix;

[0184] (c) washing non-binding proteins from the nuclear extract from the matrix;

[0185] (d) eluting bound proteins from the matrix with excess double stranded DNA corresponding to sequences found in the promoter region of the β3 gene;

[0186] where the eluted proteins from step (d) are nuclear factors involved in β3 gene transcription regulation.

[0187] In particular embodiments, the method further comprises separating the eluted proteins from step (d) on a gel and staining the gel to test for purity of the eluted proteins.

[0188] In particular embodiments, the method further comprises sequencing the proteins that have been separated on the gel.

[0189] In particular embodiments, the method further comprises immunological analysis of the proteins that have been separated on the gel with antibodies directed towards known transcription factors to identify the eluted proteins by western blot or immunoprecipitation.

[0190] In particular embodiments, the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim **18** or **21**.

[0191] In a different approach, cDNAs encoding the transcription factors that bind SEQ. ID. NO.:20; positions 1-17, 436 can be cloned by several methods. In one version, the minimal DNA sequence is radiolabeled and used to screen an expression library made from tissues or cell lines that endogenously express the β3 gene. Phage containing cDNA encoding the transcription factor are induced to express fusion proteins that target the transcription factor to its surface. Such phage plaques are identified by their ability to bind radiolabeled DNA sequences containing the minimal DNA sequence.

[0192] Accordingly, the present invention provides a method of identifying clones encoding nuclear factors involved in β3 gene transcription regulation by cloning comprising:

[0193] (a) screening an expression library with radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436)

[0194] (b) determining which clones of the library bind the radiolabeled fragments of double stranded DNA;

[0195] (c) amplifying and sequencing the clones of step (b).

[0196] In particular embodiments, the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim **18** or **21**.

[0197] Another cloning approach involves phage expressing transcription factor fusion proteins at their surface. In this approach, the minimal DNA sequence is linked to a matrix. A phage expression library is then passed over the matrix and washed. Only phage containing the transcription factor bind the matrix. Bound phage are eluted with excess minimal DNA sequence and purified. cDNA encoding the transcription factor is then isolated from the phage and sequenced.

[0198] Accordingly, the present invention provides a method of identifying nuclear factors involved in β3 gene transcription regulation by cloning comprising:

[0199] (a) attaching fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) to a stable matrix;

[0200] (b) incubating phage expressing cDNA encoded fusion proteins at their surface with the matrix;

[0201] (c) removing phage that do not bind to the matrix by washing;

[0202] (d) eluting phage bound to the matrix with excess fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene;

[0203] where the phage eluted in step (d) encode nuclear factors involved in β3 gene transcription regulation.

[0204] In particular embodiments, the DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim **18** or **21**.

[0205] In particular embodiments, the phage eluted at step (d) are amplified and sequenced.

[0206] A separate transcription factor cloning approach is the yeast 'one-hybrid' method (available in kit form from Clontech). In this method, yeast strains are made that contain several copies (three suggested) of the minimal element upstream of a reporter. A cDNA library is made such that each vector contains a cDNA that will be expressed as a fusion protein with the transcription activation domain of a yeast promoter. Thus, any fusion protein that specifically binds the DNA of interest will induce expression of the reporter protein. The vector containing the cDNA is then isolated from the yeast and sequenced.

[0207] Accordingly, the present invention provides a method of identifying nuclear factors involved in β3 gene transcription regulation by cloning comprising:

[0208] (a) constructing a yeast strain that contains a few to several copies of a fragment of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) preceding a cDNA encoding a reporter protein;

[0209] (b) constructing a cDNA library from cells in a vector that allows formation of fusion proteins encoded by the inserted cDNA and a transcription activation domain;

[0210] (c) transforming the library of (b) into the yeast strain of (a) and isolating colonies of yeast displaying expression of the reporter protein.

[0211] In particular embodiments, the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim **18** or **21**.

[0212] In particular embodiments, the method further comprises purifying the vectors from the isolated colonies and sequencing the cDNA in the vectors.

[0213] Since transcription factors often are able to recognize more than one specific nucleotide sequence, variations of sequences identified as minimal promoters, enhancers or repressors necessary for transcriptional regulation of the β3 gene in SEQ. ID. NO.:20; positions 1-17,436, that can be bound by transcription factors as detected in the above described assays are intended to be included in the present invention.

[0214] Identification of nucleotide sequences involved in transcriptional regulation of β3 gene expression by the methods described above allows for the development of assays that can be used to screen collections of substances to identify those substances that enhance or inhibit transcription of the β3 gene. Fragments of the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) that have been shown to be involved in transcriptional regulation are linked to the coding sequence of a reporter gene in a suitable vector and are then transferred to appropriate cells. The abundance of the reporter protein in the cells is determined. The cells are then exposed to compounds that are suspected of being capable of enhancing or inhibiting the rate of transcription of the β3 gene. If the compound actually is capable of enhancing the rate of transcription of the β3 gene, then the abundance of the reporter protein will be increased when the cells are exposed to the compound. Conversely, if the compound actually is capable of inhibiting the rate of transcription of the β3 gene, then the abundance of the reporter protein will be decreased when the cells are exposed to the compound.

[0215] Accordingly, the present invention provides a method of identifying substances that enhance or inhibit the rate of transcription of the β3 gene comprising:

[0216] (a) constructing a promoter-reporter vector such that fragments of the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) precede the coding cDNA sequence of a reporter gene which encodes a reporter protein;

[0217] (b) transfecting the vector into cells and measuring the abundance of the reporter protein encoded by the vector in the presence and absence of a compound;

[0218] where (1) if the presence of the compound decreases the abundance of the reporter protein, then the compound is a substance that inhibits the rate of transcription of the β3 gene; (2) if the presence of the compound increases the abundance of the reporter protein, then the compound is a substance that enhances the rate of transcription of the β3 gene.

[0219] In particular embodiments, the method further comprises a control in which the effect of the compound on the abundance of the reporter protein in control cells is measured, where the control cells are cells that are essentially the same as the cells of step (b) except that the control cells have been transfected with a vector that lacks fragments of the promoter region of the β3 gene.

[0220] While the above-described methods are explicitly directed to testing whether "a" substance is an activator or inhibitor of the transcription the β3 gene or the function of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, it will be clear to one skilled in the art that such methods can be adapted to test collections of substances, e.g., combinatorial libraries, to determine whether any members of such collections are activators or inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Accordingly, the use of collections of substances, or individual members of such collections, as the substance in the above-described methods is within the scope of the present invention. In particular, it is envisioned that libraries that have been designed to incorporate chemical structures that are known to be associated with potassium ion channel modulation, e.g., dihydrobenzopyran libraries for potassium channel activators (International Patent Publication WO 95/30642) or biphenyl-derivative libraries for potassium channel inhibitors (International Patent Publication WO 95/04277) will be especially suitable.

[0221] The present invention includes pharmaceutical compositions comprising activators or inhibitors of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins that have been identified by the herein-described methods as well as activators or inhibitors of β3 gene transcription. The activators or inhibitors are generally combined with pharmaceutically acceptable carriers to form pharmaceutical compositions. Examples of such carriers and methods of formulation of pharmaceutical compositions

containing activators or inhibitors and carriers can be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain a therapeutically effective amount of the activators or inhibitors.

[0222] Therapeutic or prophylactic compositions are administered to an individual in amounts sufficient to treat or prevent conditions where human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein activity is abnormal. The effective amount can vary according to a variety of factors such as the individual's condition, weight, gender, and age. Other factors include the mode of administration. The appropriate amount can be determined by a skilled physician. Generally, an effective amount will be from about 0.01 to about 1,000, preferably from about 0.1 to about 250 and even more preferably from about 1 to about 50 mg per adult human per day.

[0223] Compositions can be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents can be desirable.

[0224] The compositions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compositions can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they can also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

[0225] Compositions can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three, four or more times daily. Furthermore, compositions can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

[0226] The dosage regimen utilizing the compositions is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal, hepatic and cardiovascular function of the patient; and the particular composition thereof employed. A physician of ordinary skill can readily determine and prescribe the effective amount of the composition required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of composition within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the composition's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a composition.

[0227] The inhibitors and activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, or inhibitors and activators of β3 subunit transcription will be useful for treating a variety of diseases involving excessive or insufficient calcium sensitive potassium channel activity. Accordingly, the present invention includes a method of treating asthma, diabetes, glaucoma, pregnant human myometrium, cerebral ischemia, and conditions where stimulation of neurotransmitter release is desired such as Alzheimer's disease and stimulation of damaged nerves by administering to a patient a therapeutically effective amount of a substance that is an activator or an inhibitor of a calcium sensitive potassium channel containing a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein, or an activator or an inhibitor of β3 subunit transcription.

[0228] The modulators of channel function or transcription activity of the present invention are also expected to be useful in conditions where currently marketed inhibitors of potassium channels such as glyburide, glipizide, and tolbutamide are useful, e.g., as antidiabetic agents. Calcium sensitive potassium channels contribute to the repolarization, and thus the de-excitation, of neurons. Thus, inhibitors of calcium sensitive potassium channels are expected to act as agents that tend to keep neurons in a depolarized, excited state. Many diseases, such as depression and memory disorders are thought to result from the impairment of neurotransmitter release. As agents that contribute to neuronal excitability, the inhibitors of the present invention are expected to useful in the treatment of such diseases since they will contribute to neuronal excitation and thus stimulate the release of neurotransmitters.

[0229] The activators of the present invention should be useful in conditions where it is desirable to decrease neuronal activity. Such conditions include, e.g., excessive smooth muscle tone, angina, asthma, hypertension, incontinence, pre-term labor, migraine, cerebral ischemia, and Irritable Bowel Syndrome.

[0230] The calcium sensitive potassium channel subunits of the present invention are useful in conjunction with screens designed to identify activators and inhibitors of other ion channels. When screening compounds in order to identify potential pharmaceuticals that specifically interact with a target ion channel, it is necessary to ensure that the compounds identified are as specific as possible for the target ion channel. To do this, it is necessary to screen the compounds against as wide an array as possible of ion channels that are similar to the target ion channel. Thus, in order to find compounds that are potential pharmaceuticals that interact with ion channel A, it is not enough to ensure that the compounds interact with ion channel A (the "plus target") and produce the desired pharmacological effect through ion channel A. It is also necessary to determine that the compounds do not interact with ion channels B, C, D, etc. (the "minus targets"). In general, as part of a screening program, it is important to have as many minus targets as possible (see Hodgson, 1992, Bio/Technology 10:973-980, at 980). Human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, DNA encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins, and recombinant cells that have been engineered to express human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins have utility in that they can be used as "minus targets" in screens designed to identify compounds that specifically interact with other ion channels. For example, Wang et al.,

1998, Science 282:1890-1893 have shown that KCNQ2 and KCNQ3 form a heteromeric potassium ion channel know as the "M-channel." The M-channel is an important target for drug discovery since mutations in KCNQ2 and KCNQ3 are responsible for causing epilepsy (Biervert et al., 1998, Science 279:403406; Singh et al., 1998, Nature Genet. 18:25-29; Schroeder et al., Nature 1998, 396:687-690). A screening program designed to identify activators or inhibitors of the M-channel would benefit greatly by the use of potassium channels comprising human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins as minus targets.

[0231] The present invention also includes antibodies to the human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins. Such antibodies may be polyclonal antibodies or monoclonal antibodies. The antibodies of the present invention can be raised against the entire human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins or against suitable antigenic fragments of the subunit proteins that are coupled to suitable carriers, e.g., serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. See, e.g., Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer Applications in the Biosciences) 4:181-186.

[0232] For the production of polyclonal antibodies, human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins or antigenic fragments, coupled to a suitable carrier, are injected on a periodic basis into an appropriate non-human host animal such as, e.g., rabbits, sheep, goats, rats, mice or chickens. The animals are bled periodically (or eggs collected) and sera obtained are tested for the presence of antibodies to the injected subunit or antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

[0233] For the production of monoclonal antibodies, human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins or antigenic fragments, coupled to a suitable carrier, are injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of monoclonal antibodies, see *Antibodies: A Laboratory Manual*, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

[0234] Gene therapy may be used to introduce human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins into the cells of target organs. Nucleotides encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, lentivirus, and polio virus based vectors. Alternatively, nucleotides encoding human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for ex vivo as well as in vivo gene therapy. Gene therapy with human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins will be particularly useful for the treatment of diseases where it is beneficial to elevate calcium sensitive potassium channel activity. cDNAs encoding mutant calcium sensitive potassium channel subunits, that display a dominant negative phenotype, may be particularly useful for gene therapy treatment of diseases where it is beneficial to decrease calcium sensitive potassium channel activity.

[0235] The present invention includes processes for cloning orthologues of human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits from non-human species. In general, such processes include preparing a PCR primer or a hybridization probe based upon SEQ. ID. NO.:1, 3, 5, 7, 9, or 20 that can be used to amplify a fragment containing the non-human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit (in the case of PCR) from a suitable DNA preparation or to select a cDNA or genomic clone containing the non-human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit from a suitable library. A preferred embodiment of this process is a process for cloning the calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit from mouse.

[0236] By providing DNA encoding mouse calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunits, the present invention allows for the generation of an animal model of human diseases in which calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit activity is abnormal. Such animal models can be generated by making transgenic "knockout" or "knockin" mice containing altered calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit genes. Knockout mice can be generated in which portions of the mouse calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit gene have been deleted. Knockin mice can be generated in which mutations that have been shown to lead to human disease are introduced into the mouse gene. Such knockout and knockin mice will be valuable tools in the study of the relationship between calcium sensitive potassium channels and disease and will provide important model systems in which to test potential pharmaceuticals or treatments for human diseases involving calcium sensitive potassium channels.

[0237] Accordingly, the present invention includes a method of producing a transgenic mouse comprising:

[0238] (a) designing PCR primers or an oligonucleotide probe based upon SEQ. ID. NO.:1, 3, 5, 7, 9 or 20 for use in cloning the mouse calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit gene or cDNA;

[0239] (b) using the PCR primers or the oligonucleotide probe to clone at least a portion of the mouse calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit gene or cDNA, the portion being large enough to use in making a transgenic mouse;

[0240] (c) producing a transgenic mouse having at least one copy of the mouse calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit gene altered from its native state.

[0241] Methods of producing knockout and knockin mice are well known in the art. One method involves the use of gene-targeted ES cells in the generation of gene-targeted transgenic knockout mice and is described in, e.g., Thomas et al., 1987, Cell 51:503-512, and is reviewed elsewhere (Frohman et al., 1989, Cell 56:145-147; Capecchi, 1989, Trends in Genet. 5:70-76; Baribault et al., 1989, Mol. Biol. Med. 6:481-492).

[0242] Techniques are available to inactivate or alter any genetic region to virtually any mutation desired by using targeted homologous recombination to insert specific changes into chromosomal genes. Generally, use is made of a "targeting vector," i.e., a plasmid containing part of the genetic region it is desired to mutate. By virtue of the homology between this part of the genetic region on the plasmid and the corresponding genetic region on the chromosome, homologous recombination can be used to insert the plasmid into the genetic region, thus disrupting the genetic region. Usually, the targeting vector contains a selectable marker gene as well.

[0243] In comparison with homologous extrachromosomal recombination, which occurs at frequencies approaching 100%, homologous plasmid-chromosome recombination was originally reported to only be detected at frequencies between 10^{-6} and 10^{-3} (Lin et al., 1985, Proc. Natl. Acad. Sci. USA 82:1391-1395; Smithies et al., 1985, Nature 317: 230-234; Thomas et al., 1986, Cell 44:419428). Nonhomologous plasmid-chromosome interactions are more frequent, occurring at levels 10^5-fold (Lin et al., 1985, Proc. Natl. Acad. Sci. USA 82:1391-1395) to 10^2-fold (Thomas et al., 1986, Cell 44:419-428) greater than comparable homologous insertion.

[0244] To overcome this low proportion of targeted recombination in murine ES cells, various strategies have been developed to detect or select rare homologous recombinants. One approach for detecting homologous alteration events uses the polymerase chain reaction (PCR) to screen pools of transformant cells for homologous insertion, followed by screening individual clones (Kim et al., 1988, Nucleic Acids Res. 16:8887-8903; Kim et al., 1991, Gene 103:227-233). Alternatively, a positive genetic selection approach has been developed in which a marker gene is constructed which will only be active if homologous insertion occurs, allowing these recombinants to be selected directly (Sedivy et al., 1989, Proc. Natl. Acad. Sci. USA 86:227-231). One of the most powerful approaches developed for selecting homologous recombinants is the positive-negative selection (PNS) method developed for genes for which no direct selection of the alteration exists (Mansour et al., 1988, Nature 336:348-352; Capecchi, 1989, Science 244:1288-1292; Capecchi, 1989, Trends in Genet. 5:70-76). The PNS method is more efficient for targeting genes which are not expressed at high levels because the marker gene has its own promoter. Nonhomologous recombinants are selected against by using the Herpes Simplex virus thymidine kinase (HSV-TK) gene and selecting against its nonhomologous insertion with herpes drugs such as gancyclovir (GANC) or FHAU (1-(2-deoxy 2-fluoro-B-D-arabinofluranosyl)-5-iodouracil). By this counter-selection, the percentage of homologous recombinants in the surviving transformants can be increased.

[0245] Other methods of producing transgenic mice involve microinjecting the male pronuclei of fertilized eggs. Such methods are well known in the art.

[0246] The present invention includes a transgenic, nonhuman animal in which the animal's genome contains DNA encoding at least a portion of a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit.

[0247] The following non-limiting examples are presented to better illustrate the invention.

EXAMPLE 1

[0248] Identification of the Human Calcium Sensitive Potassium Channel β2, β3a, β3b, β3c, or β3d Subunits and cDNA Cloning

[0249] DNA sequence encoding the β1 subunit was used to search the GenBank database for homologous sequences encoding novel subunits. This search yielded an EST with similarity to β1 (AA904191). A cDNA encoding the EST was purchased (Genome Systems) and sequenced in both directions. Synthetic oligonucleotide primers (SEQ. ID. NOs.:12 and 13) were used to amplify the coding region and a small portion of the 3' untranslated region (UTR) of this gene (β2). The coding region was then subcloned into a modified vector (pSP64T) containing an expanded polylinker between the 5' and 3' translation enhancer sequences (MVpl(+)).

[0250] The sequence of β2 was then used to search the GenBank database for additional novel beta subunits. The sequences from identified EST's were then used to search the database again. Several EST's were obtained in this iterative approach: AA195381, AA236930, AA236968, AA279911, AA761761, AA934876, AA195511, AA917510. The alignment of these sequences suggested they encoded the C-terminal portion of a novel β subunit, here designated β3. Available cDNAs encoding these ESTs were purchased (Genome Systems) and sequenced in both directions. None of these clones encoded full length protein based on the lack of 5' in-frame stop codons and amino acid alignments only to the middle of the first transmembrane segments of β1 and β2.

[0251] Unique and conserved portions of the individual subunits were used separately to search the databases for genomic sequences encoding these transcripts. A single 180 kilobase fragment of unidentified genomic sequence was identified using β3a, β3b and β3c specific fragments (GenBank accession number AC007823, version 2). Later versions of this entry contained a 40.4 kilobase contiguous fragment that contained all three specific fragments in the following order β3a, β3b and β3c. β3c is contiguous with the 5' end of the core sequence. See **FIG. 8**.

[0252] A synthetic oligo, 5'-TTT ACA TTG TTA GTT TGC AGA CAG G-3' (SEQ. ID. NO.:19), annealing 3' of the β3 stop codon was used in a 5' RACE reaction as described in Clontech's Marathon Ready Spleen cDNA kit (catalog # 7412-1). This reaction yielded multiple products of varying sizes. Several fragments separated by electrophoresis were extracted from gel slices and cloned. Three distinct subunits were identified (β3a, β3b and β3c) in this manner.

[0253] To ensure novel subunits were not overlooked, the unfractionated product of the PCR amplification reaction

was cloned directly into a TA cloning vector (pCR2.1, Invitrogen), without any attempt to isolate specific fragments. Colonies were then screened using a probe derived from EST AA761761 by the 'colony filter hybridization protocol' as described in *Current Protocols in Molecular Biology*, sections 6.1.1 and 6.3.1. DNA was prepared from hybridizing colonies. cDNAs with restriction digest patterns distinct from the original clones were sequenced in both directions. The open reading frames were determined and amplified using synthetic oligonucleotide primers (SEQ. ID. NOs.:14 through 18), and subcloned into MVpl(+). One additional unique subunit was identified: β3d.

EXAMPLE 2

[0254] Analysis of Expression of Human Calcium Sensitive Potassium Channel β2, β3a, β3b, β3c, or β3d Subunits

[0255] Northern blot analysis: Northern blots containing poly(A+)-RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testes, ovary, small intestine, colon, and peripheral blood leukocytes were purchased from Clontech, Palo Alto, Calif. The blots were probed with ^{32}P-labeled, randomly primed cDNA probes from β2 (nucleotides 268 to 1080 of SEQ. ID. NO.:1), β3a (nucleotides 70 to 384 of SEQ. ID. NO.:3), β3b (nucleotides 463 to 797 of SEQ. ID. NO.:5), and β3c/d (nucleotides 311 to 912 of SEQ. ID. NO.:7). The hybridization was carried out in 5×SSPE, 10× Denhardts solution, 50% Formamide, 2% SDS, 100 ug/mil salmon sperm DNA at 42° C. overnight. The washes were carried out stepwise in 2×SSC, 0.05% SDS at 42° C. for 40 minutes, followed by 1×SSC, 0.05% SDS at 50° C. for 40 minutes. High stringency washes were carried out at 0.1 SSC, 0.05% SDS at 65° C. for 40 minutes. Hybridization was detected either by exposure of the washed blots to X-ray film or by electronic detection using a phosphorimager.

[0256] Electrophysiological analysis: cRNAs were synthesized in vitro from plasmids encoding human Slowpoke a or the β2, β3a, β3b, β3c, or β3d subunits and injected into *Xenopus* oocytes (1.5 ng/oocyte of α subunit RNA+/−β subunit RNA at 1, 5, or 10× molar excess). Calcium sensitive potassium currents were recorded in inside-out patches. Recordings were performed under ionic conditions of symmetrical potassium. The standard pipette and bath solutions contained 116 mM potassium gluconate, 4 mM potassium chloride, 10 mM HEPES, pH 7.2. $CaCl_2$ was added to the bath solution to give final concentrations of free ionized calcium of 3-30 μM, taking into account the stability constant for calcium gluconate (15.9 M^{-1}). Currents were recorded using an EPC-7 amplifier (HEKA). The pClamp6.0 program (Axon Instruments) was used to generate voltage-clamp commands for data acquisition, and for analysis. NP_o-voltage relations were determined at 3, 10 and 30 μM bath calcium using two methods: (1) calculation of macroscopic conductance from peak or steady-state currents at test potentials (−80 to 80 mV), or (2) measurement or calculation of tail currents peaks (−80 mV) at test potentials. Boltzmann functions were fit to the data and used to derive the half-maximal activation parameter ($V_{1/2}$). Maximal inactivation parameters (30 μM Ca^{2+} and 80 mV) were calculated from current traces or averaged current traces. Inactivation rates were determined from single exponential fits. Fractional non-inactivating current was calculated as steady-state/peak current; fractional inactivating current was estimated as peak current minus steady-state current divided by peak current.

[0257] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

[0258] Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

SEQUENCE LISTING

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ctgtggggcc cttccagaga aatgtactga aaaagtctac gcaatgtctg ggatttgcta 180

aacaatacct ggaaagcaga caggtttttt tgccattcct ccaggacatc caccataagg 240

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taccctgcc tccaggtgta cgttaacctg acttcttccg gggaaaagct cctcctctac 660

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cacttctcct gctattctga cccagaagga aaccagaaga gtgttatcct aaccaaactc 840

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caacggatca atagataaat gcaaaaatgg ataaaataat ttttgttaaa gctcaaatac 1020

tgttttcttt cattcttcac caaagaacct taagtttgta acgtgcagtc tgttatgagt 1080

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            20 25 30

Lys Arg Lys Thr Val Thr Ala Leu Lys Ala Gly Glu Asp Arg Ala Ile
        35 40 45

Leu Leu Gly Leu Ala Met Met Val Cys Ser Ile Met Met Tyr Phe Leu
    50 55 60

Leu Gly Ile Thr Leu Leu Arg Ser Tyr Met Gln Ser Val Trp Thr Glu
65 70 75 80

Glu Ser Gln Cys Thr Leu Leu Asn Ala Ser Ile Thr Glu Thr Phe Asn
                85 90 95

Cys Ser Phe Ser Cys Gly Pro Asp Cys Trp Lys Leu Ser Gln Tyr Pro
            100 105 110

Cys Leu Gln Val Tyr Val Asn Leu Thr Ser Ser Gly Glu Lys Leu Leu
        115 120 125

Leu Tyr His Thr Glu Glu Thr Ile Lys Ile Asn Gln Lys Cys Ser Tyr
    130 135 140

Ile Pro Lys Cys Gly Lys Asn Phe Glu Glu Ser Met Ser Leu Val Asn
145 150 155 160

Val Val Met Glu Asn Phe Arg Lys Tyr Gln His Phe Ser Cys Tyr Ser
                165 170 175

Asp Pro Glu Gly Asn Gln Lys Ser Val Ile Leu Thr Lys Leu Tyr Ser
            180 185 190

Ser Asn Val Leu Phe His Ser Leu Phe Trp Pro Thr Cys Met Met Ala
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tctctaggaa ggcgggggcc ttctaatggc tgcagctgcg ctgggggctg ggggctcccg 240

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tgccttcacc tgtggtgtgc actgccacgg tcaggggaag tacccgtgtc ttcaggtgtt 720

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Thr Asp Tyr Ser Asp Gly Asp Pro Leu Asp Val His Lys Arg Leu Pro
35 40 45

Ser Ser Thr Gly Glu Asp Arg Ala Val Met Leu Gly Phe Ala Met Met
50 55 60

Gly Phe Ser Val Leu Met Phe Phe Leu Leu Gly Thr Thr Ile Leu Lys
65 70 75 80

Pro Phe Met Leu Ser Ile Gln Arg Glu Glu Ser Thr Cys Thr Ala Ile

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               85                  90                  95

His Thr Asp Ile Met Asp Asp Trp Leu Asp Cys Ala Phe Thr Cys Gly
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Val His Cys His Gly Gln Gly Lys Tyr Pro Cys Leu Gln Val Phe Val
        115                 120                 125

Asn Leu Ser His Pro Gly Gln Lys Ala Leu Leu His Tyr Asn Glu Glu
    130                 135                 140

Ala Val Gln Ile Asn Pro Lys Cys Phe Tyr Thr Pro Lys Cys His Gln
145                 150                 155                 160

Asp Arg Ser Asp Leu Leu Asn Ser Ala Leu Asp Ile Lys Glu Phe Phe
                165                 170                 175

Asp His Lys Asn Gly Thr Pro Phe Ser Cys Phe Tyr Ser Pro Ala Ser
            180                 185                 190

Gln Ser Glu Asp Val Ile Leu Ile Lys Lys Tyr Asp Gln Met Ala Ile
        195                 200                 205

Phe His Cys Leu Phe Trp Pro Ser Leu Thr Leu Leu Gly Gly Ala Leu
    210                 215                 220

Ile Val Gly Met Val Arg Leu Thr Gln His Leu Ser Leu Leu Cys Glu
225                 230                 235                 240

Lys Tyr Ser Thr Val Val Arg Asp Glu Val Gly Gly Lys Val Pro Tyr
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gaagagcagg taaagtctac tgacagaaag tcagcagtgg aagcccaaaa cgaggtgact    180

gaaaatccaa aacagaaaat tgcagcagaa agcagtgaaa atgctgattg tccagagaat    240

cctaaaatga agttggatgg aaaacttgac caagaaggca atgatgtaaa aacagcagct    300

gaggaggtac tagctggtag agacacatta gattttgagg atgtcacagt tcaatcatca    360

ggcccgaggg ctggtggtga agaattagat gaaggtgttg caaaagataa tgctaaaata    420

gctggtgcca cttaaagcaa tcctgaagaa ccagagagtg aagatgcaga tcactgcacc    480

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atccacacag atatcatgga cgactggctg gactgtgcct tcacctgtgg tgtgcactgc   1080

cacggtcagg ggaagtaccc gtgtcttcag gtgtttgtga acctcagcca tccaggtcag   1140

aaagctctcc tacattataa tgaagaggct gtccagataa atcccaagtg cttttacaca   1200

cctaagtgcc accaagatag aaatgatttg ctcaacagtg ctctggacat aaaagaattc   1260

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tatatagaac agcatcagtt caaactgtgc attatgagga ggagcaaagg aagagcagag   1560

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Glu Asp Arg Ala Val Met Leu Gly Phe Ala Met Met Gly Phe Ser Val
        35                  40                  45

Leu Met Phe Phe Leu Leu Gly Thr Thr Ile Leu Lys Pro Phe Met Leu
    50                  55                  60

Ser Ile Gln Arg Glu Glu Ser Thr Cys Thr Ala Ile His Thr Asp Ile
65                  70                  75                  80

Met Asp Asp Trp Leu Asp Cys Ala Phe Thr Cys Gly Val His Cys His
                85                  90                  95

Gly Gln Gly Lys Tyr Pro Cys Leu Gln Val Phe Val Asn Leu Ser His
            100                 105                 110

Pro Gly Gln Lys Ala Leu Leu His Tyr Asn Glu Glu Ala Val Gln Ile
        115                 120                 125

Asn Pro Lys Cys Phe Tyr Thr Pro Lys Cys His Gln Asp Arg Asn Asp
    130                 135                 140

Leu Leu Asn Ser Ala Leu Asp Ile Lys Glu Phe Phe Asp His Lys Asn
145                 150                 155                 160

Gly Thr Pro Phe Ser Cys Phe Tyr Ser Pro Ala Ser Gln Ser Glu Asp
                165                 170                 175

Val Ile Leu Ile Lys Lys Tyr Asp Gln Met Ala Ile Phe His Cys Leu
            180                 185                 190

Phe Trp Pro Ser Leu Thr Leu Leu Gly Gly Ala Leu Ile Val Gly Met
        195                 200                 205

Val Arg Leu Thr Gln His Leu Ser Leu Leu Cys Glu Lys Tyr Ser Thr
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Val Val Arg Asp Glu Val Gly Gly Lys Val Pro Tyr Ile Glu Gln His
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Gln Phe Lys Leu Cys Ile Met Arg Arg Ser Lys Gly Arg Ala Glu Lys
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gatctctaca gacttgcatg aggcaacatt tcttaggctt gtttgctaca atatctttaa    720

aaatacttga ttacacatca ctttagctta tttagatgga cttttcacca agctctgaac    780

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Tyr Ser Asp Gly Asp Pro Leu Asp Val His Lys Arg Leu Pro Ser Ser
        35   40   45

Thr Gly Glu Asp Arg Ala Val Met Leu Gly Phe Ala Met Met Gly Phe
    50   55   60

Ser Val Leu Met Phe Phe Leu Leu Gly Thr Thr Ile Leu Lys Pro Phe
65   70   75   80

Met Leu Ser Ile Gln Arg Glu Glu Ser Thr Cys Thr Ala Ile His Thr
                85   90   95

Asp Ile Met Asp Asp Trp Leu Asp Cys Ala Phe Thr Cys Gly Val His
            100   105   110

Cys His Gly Gln Gly Lys Tyr Pro Cys Leu Gln Val Phe Val Asn Leu
        115   120   125

Ser His Pro Gly Gln Lys Ala Leu Leu His Tyr Asn Glu Glu Ala Val
    130   135   140

Gln Ile Asn Pro Lys Cys Phe Tyr Thr Pro Lys Cys His Gln Asp Arg
145   150   155   160

Ser Asp Leu Leu Asn Ser Ala Leu Asp Ile Lys Glu Phe Phe Asp His
                165   170   175

Lys Asn Gly Thr Pro Phe Ser Cys Phe Tyr Ser Pro Ala Ser Gln Ser
            180   185   190

Glu Asp Val Ile Leu Ile Lys Lys Tyr Asp Gln Met Ala Ile Phe His
        195   200   205

Cys Leu Phe Trp Pro Ser Leu Thr Leu Leu Gly Gly Ala Leu Ile Val
    210   215   220

Gly Met Val Arg Leu Thr Gln His Leu Ser Leu Leu Cys Glu Lys Tyr
225   230   235   240

Ser Thr Val Val Arg Asp Glu Val Gly Gly Lys Val Pro Tyr Ile Glu
                245   250   255

Gln His Gln Phe Lys Leu Cys Ile Met Arg Arg Ser Lys Gly Arg Ala
            260   265   270

Glu Lys Ser
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<210> SEQ ID NO 9
<211> LENGTH: 1296
<212> TYPE: DNA
<213> ORGANISM: Human

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<210> SEQ ID NO 10
<211> LENGTH: 279
<212> TYPE: PRT
<213> ORGANISM: Amino Acid

<400> SEQUENCE: 10

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Ile Leu Leu Thr Arg His Arg Thr Ala Phe Pro Ala Ser Gly Lys Lys
            20                  25                  30

Arg Glu Thr Asp Tyr Ser Asp Gly Asp Pro Leu Asp Val His Lys Arg
        35                  40                  45

Leu Pro Ser Ser Thr Gly Glu Asp Arg Ala Val Met Leu Gly Phe Ala
    50                  55                  60

Met Met Gly Phe Ser Val Leu Met Phe Phe Leu Leu Gly Thr Thr Ile
65                  70                  75                  80

Leu Lys Pro Phe Met Leu Ser Ile Gln Arg Glu Glu Ser Thr Cys Thr
                85                  90                  95

Ala Ile His Thr Asp Ile Met Asp Asp Trp Leu Asp Cys Ala Phe Thr
            100                 105                 110

Cys Gly Val His Cys His Gly Gln Gly Lys Tyr Pro Cys Leu Gln Val
        115                 120                 125

Phe Val Asn Leu Ser His Pro Gly Gln Lys Ala Leu Leu His Tyr Asn
    130                 135                 140

Glu Glu Ala Val Gln Ile Asn Pro Lys Cys Phe Tyr Thr Pro Lys Cys
145                 150                 155                 160
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His Gln Asp Arg Asn Asp Leu Leu Asn Ser Ala Leu Asp Ile Lys Glu
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Phe Phe Asp His Lys Asn Gly Thr Pro Phe Ser Cys Phe Tyr Ser Pro
            180                 185                 190

Ala Ser Gln Ser Glu Asp Val Ile Leu Ile Lys Lys Tyr Asp Gln Met
        195                 200                 205

Ala Ile Phe His Cys Leu Phe Trp Pro Ser Leu Thr Leu Leu Gly Gly
    210                 215                 220

Ala Leu Ile Val Gly Met Val Arg Leu Thr Gln His Leu Ser Leu Leu
225                 230                 235                 240

Cys Glu Lys Tyr Ser Thr Val Val Arg Asp Glu Val Gly Gly Lys Val
                245                 250                 255

Pro Tyr Ile Glu Gln His Gln Phe Lys Leu Cys Ile Met Arg Arg Ser
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Lys Gly Arg Ala Glu Lys Ser
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<210> SEQ ID NO 11
<211> LENGTH: 191
<212> TYPE: PRT
<213> ORGANISM: Amino Acid

<400> SEQUENCE: 11

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Ile Leu Val Thr Thr Val Leu Pro Leu Tyr Gln Lys Ser Val Trp Thr
        35                  40                  45

Gln Glu Ser Lys Cys His Leu Ile Glu Thr Asn Ile Arg Asp Gln Glu
    50                  55                  60

Glu Leu Lys Gly Lys Lys Val Pro Gln Tyr Pro Cys Leu Trp Val Asn
65                  70                  75                  80

Val Ser Ala Ala Gly Arg Trp Ala Val Leu Tyr His Thr Glu Asp Thr
                85                  90                  95

Arg Asp Gln Asn Gln Gln Cys Ser Tyr Ile Pro Gly Ser Val Asp Asn
            100                 105                 110

Tyr Gln Thr Ala Arg Ala Asp Val Glu Lys Val Arg Ala Lys Phe Gln
        115                 120                 125

Glu Gln Gln Val Phe Tyr Cys Phe Ser Ala Pro Arg Gly Asn Glu Thr
    130                 135                 140

Ser Val Leu Phe Gln Arg Leu Tyr Gly Pro Gln Ala Leu Leu Phe Ser
145                 150                 155                 160

Leu Phe Trp Pro Thr Phe Leu Leu Thr Gly Gly Leu Leu Ile Ile Ala
                165                 170                 175

Met Val Lys Ser Asn Gln Tyr Leu Ser Ile Leu Ala Ala Gln Lys
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<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Human

<400> SEQUENCE: 12

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<400> SEQUENCE: 13

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<400> SEQUENCE: 14

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<211> LENGTH: 24
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<400> SEQUENCE: 15

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<400> SEQUENCE: 16

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<211> LENGTH: 24
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<400> SEQUENCE: 17

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<210> SEQ ID NO 18
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Human

<400> SEQUENCE: 18

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<210> SEQ ID NO 19
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic oligo

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<210> SEQ ID NO 20

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<211> LENGTH: 48000
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<223> OTHER INFORMATION: n = A,T,C or G

<400> SEQUENCE: 20

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1. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding a human calcium sensitive potassium channel subunit protein designated β2, wherein said protein comprises the amino acid sequence as set forth in SEQ ID NO:2.

2. (canceled)

3. The isolated nucleic acid molecule of claim 1 comprising a nucleotide sequence as set forth in SEQ ID NO:1.

4. The isolated nucleic acid molecule of claim 2, wherein said nucleotide sequence comprises a coding portion from nucleotide position 271 to nucleotide 978 of SEQ ID NO: 1.

5. (canceled)

6. An expression vector comprising the nucleic acid molecule of claim 1.

7. A recombinant host cell comprising the nucleic acid molecule of claim 1.

8. An isolated and substantially pure human calcium sensitive potassium channel subunit protein comprising an amino acid sequence as set forth in SEQ ID NO:2.

9. (canceled)

10. (canceled)

11. (canceled)

12. An isolated and substantially pure polypeptide having at least 80% sequence identity to the protein of claim 8 when measured by BLAST or FASTA.

13. An antibody that binds specifically to a human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit protein; or that binds specifically to the β3 subunit family of proteins by binding to the conserved core.

14. (canceled)

15. A method for identifying substances that bind to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) providing cells expressing a calcium sensitive potassium channel containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(b) exposing the cells to a substance that is not known to bind calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(c) determining the amount of binding of the substance to the cells;

(d) comparing the amount of binding in step (c) to the amount of binding of the substance to control cells where the control cells are substantially identical to the cells of step (a) except that the control cells do not express human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

where if the amount of binding in step (c) is greater than the amount of binding of the substance to control cells, then the substance binds to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

16. A method of identifying substances that bind calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and thus are likely to be inhibitors or activators of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) providing cells expressing calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(b) exposing the cells to a compound that is known to bind to the calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

(c) determining the amount of binding of the compound to the cells in the presence and in the absence of a substance not known to bind to calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

where if the amount of binding of the compound in the presence of the substance differs from that in the absence of the substance, then the substance binds calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins and is likely to be an inhibitor or activator of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

17. A method of identifying activators or inhibitors of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins comprising:

(a) recombinantly expressing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit

proteins or mutant human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins in a host cell so that the recombinantly expressed human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins form calcium sensitive potassium channels by forming heteromers with other calcium sensitive potassium channel subunit proteins;

(b) measuring the biological activity of the calcium sensitive potassium channels formed in step (a) in the presence and in the absence of a substance suspected of being an activator or an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins;

where a change in the biological activity of the calcium sensitive potassium channels formed in step (a) in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of calcium sensitive potassium channels containing human calcium sensitive potassium channel β2, β3a, β3b, β3c, or β3d subunit proteins.

18. A method of identifying DNA sequences in the β3 gene that promote, enhance, or repress gene transcription comprising:

(a) constructing a promoter-reporter vector such that fragments of the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) precede the coding cDNA sequence of a reporter gene which encodes a reporter protein;

(b) transfecting the vector into cells and measuring the abundance of the reporter protein encoded by the vector;

(c) comparing the abundance of the reporter protein in the cells of step (b) to the abundance of the reporter protein in cells transfected with the vector without fragments of the promoter region of the β3 gene;

where fragments of the promoter region of the β3 gene which increase the abundance of the reporter protein in the absence of other promoter elements only in cells which endogenously express β3a, β3b, β3c, or β3d subunits are promoter elements; sequences which decrease the abundance of the reporter protein in the presence of an unrelated constitutive promoter element in cells which do not endogenously express β3a, β3b, β3c, or β3d subunits are repressor elements; and sequences which increase the abundance of the reporter protein in the presence of an unrelated constitutive promoter element in cells which endogenously express β3a, β3b, β3c, or β3d subunits are enhancer elements.

19. The method of claim 18 where the vector contains promoter or enhancer sequence elements which function independently of the fragments of the promoter region of the β3 gene.

20. The method of claim 18 where the abundance of the reporter protein is normalized with respect to the fraction of transfected cells.

21. A method of identifying DNA sequences in the β3 gene that promote, enhance, or repress gene transcription comprising:

(a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) with nuclear extracts from cells; and

(b) separating the incubation on a gel;

where fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene that migrate differently in a gel ('undergo a shift') after incubation with nuclear extracts from cells are DNA sequences which bind nuclear factors which promote, enhance or repress β3 gene expression.

22. The method of claim 21 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the method of claim 18.

23. The method of claim 21 where the cells express β3a, β3b, β3c, or β3d subunits.

24. The method of claim 21 where the cells do not express β3a, β3b, β3c, or β3d subunits.

25. A method of identifying nuclear factors involved in β3 gene transcription regulation comprising:

(a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) with cloned or purified transcription factors and separating the incubation on a gel;

where factors which bind β3 gene promoter sequence elements will induce a shift in the migration of the radiolabeled DNA fragments, and are involved in β3 gene transcription regulation.

26. The method of claim 25 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

27. A method of identifying transcription factors involved in β3 gene transcription regulation comprising:

(a) incubating radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) with nuclear extracts from cells and separating the incubation on a gel;

(b) adding an antibody that specifically recognizes a single transcription factor or a family of transcription factors to the incubation of step (a), followed by separating the incubation on a gel;

where a super-shift in mobility of the double stranded DNA in step (b) as compared to step (a) indicates that a transcription factor recognized by the antibody binds the double stranded DNA.

28. A method of identifying clones encoding nuclear factors involved in β3 gene transcription regulation by cloning comprising:

(a) screening an expression library with radiolabeled fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436)

(b) determining which clones of the library bind the radiolabeled fragments of double stranded DNA;

(c) amplifying and sequencing the clones of step (b).

29. The method of claim 28 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

30. A method of identifying nuclear factors involved in β3 gene transcription regulation by cloning comprising:

(a) attaching fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) to a stable matrix;

(b) incubating phage expressing cDNA encoded fusion proteins at their surface with the matrix;

(c) removing phage that do not bind to the matrix by washing;

(d) eluting phage bound to the matrix with excess fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene;

where the phage eluted in step (d) encode nuclear factors involved in β3 gene transcription regulation.

31. The method of claim 30 where the DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

32. The method of claim 30 where the phage eluted at step (d) are amplified and sequenced.

33. A method of identifying nuclear factors involved in β3 gene transcription regulation comprising:

(a) attaching fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) to a stable matrix;

(b) incubating nuclear extracts from cells with the matrix;

(c) washing non-binding proteins from the nuclear extract from the matrix;

(d) eluting bound proteins from the matrix with excess double stranded DNA corresponding to sequences found in the promoter region of the β3 gene;

where the eluted proteins from step (d) are nuclear factors involved in β3 gene transcription regulation.

34. The method of claim 33 further comprising separating the eluted proteins from step (d) on a gel and staining the gel to test for purity of the eluted proteins.

35. The method of claim 34 further comprising sequencing the proteins that have been separated on the gel.

36. The method of claim 34 further comprising immunological analysis of the proteins that have been separated on the gel with antibodies directed towards known transcription factors to identify the eluted proteins by western blot or immunoprecipitation.

37. The method of claim 33 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

38. A method of identifying nuclear factors involved in β3 gene transcription regulation by cloning comprising:

(a) constructing a yeast strain that contains a few to several copies of a fragment of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) preceding a cDNA encoding a reporter protein;

(b) constructing a cDNA library from cells in a vector that allows formation of fusion proteins encoded by the inserted cDNA and a transcription activation domain;

(c) transforming the library of (b) into the yeast strain of (a) and isolating colonies of yeast displaying expression of the reporter protein.

39. The method of claim 38 where the fragments of double stranded DNA corresponding to sequences found in the promoter region of the β3 gene are identified by the methods of claim 18 or 21.

40. The method of claim 38 further comprising purifying the vectors from the isolated colonies and sequencing the cDNA in the vectors.

41. A method of identifying substances that enhance or inhibit the rate of transcription of the β3 gene comprising:

(a) constructing a promoter-reporter vector such that fragments of the promoter region of the β3 gene (SEQ. ID. NO.:20, nucleotides 1 to 17,436) precede the coding cDNA sequence of a reporter gene which encodes a reporter protein;

(b) transfecting the vector into cells and measuring the abundance of the reporter protein encoded by the vector in the presence and absence of a compound;

where (1) if the presence of the compound decreases the abundance of the reporter protein, then the compound is a substance that inhibits the rate of transcription of the β3 gene; (2) if the presence of the compound increases the abundance of the reporter protein, then the compound is a substance that enhances the rate of transcription of the β3 gene.

42. The method of claim 41 further comprising a control in which the effect of the compound on the abundance of the reporter protein in control cells is measured, where the control cells are cells that are essentially the same as the cells of step (b) except that the control cells have been transfected with a vector that lacks fragments of the promoter region of the β3 gene.

43. A recombinant host cell comprising a heterologous human calcium sensitive potassium channel subunit protein, wherein said calcium sensitive potassium channel subunit protein is encoded by a heterologous nucleic acid molecule comprising a sequence of nucleotides or ribonucleotides as set forth in SEQ ID NO: 1.

44. A recombinant host cell comprising a heterologous human calcium sensitive potassium channel subunit protein, wherein said calcium sensitive potassium channel subunit protein is encoded by a heterologous nucleic acid molecule comprising a sequence of nucleotides or ribonucleotides that encode the amino acid sequence as set forth in SEQ ID NO: 2.

45. A method of producing the recombinant protein of claim 8, comprising:

(a) inserting a nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO:2 into an expression vector;

(b) transferring the expression vector into a host cell;

(c) culturing the host cell under conditions appropriate for amplification of the vector and expression of the protein; and

(d) harvesting the protein.

* * * * *

专利名称(译)	编码人钙敏感性钾通道亚基蛋白的分离的核酸分子称为β2，编码的蛋白质及其用途		
公开(公告)号	US20050255559A1	公开(公告)日	2005-11-17
申请号	US11/159597	申请日	2005-06-23
[标]申请(专利权)人(译)	UEBELE VICTOR SWANSON RICHARD 刘媛 LAGRUTTA ARMANDO		
申请(专利权)人(译)	UEBELE VICTOR SWANSON RICHARD 刘渊 LAGRUTTA ARMANDO		
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IPC分类号	G01N33/50 C07K14/47 C07K14/705 C07K16/18 C12N1/15 C12N1/19 C12N1/21 C12N5/10 C12N15/09 C12N15/12 C12Q1/02 C12Q1/68 G01N33/15 G01N33/53 G01N33/559 G01N33/561 G01N33/58 C07H21/04 C12P21/06		
CPC分类号	C07K14/705		
优先权	10/031691 2002-04-18 US 60/144764 1999-07-20 US PCT/US2000/019585 2000-07-18 WO		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及编码钙敏感性钾通道亚基β2，β3a，β3b，β3c和β3d的新型人DNA序列，由DNA序列编码的蛋白质，包含DNA序列的载体，含有载体的宿主细胞，以及鉴定含有人β2，β3a，β3b，β3c或β3d亚基的钙敏感性钾通道的抑制剂和激动剂，以及β3基因转录的抑制剂和激动剂。

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   1  CTTAATCCTA TCCAAGTATG CAGTACGCTC TTGGGTCGTC TCATGAGACC CAGGGGCATG
  61  TTGGAAGAA CTGAGAGAAA GAGCAACAAA GCGGCGAGTG GTGTGAGAGG GCAGCACGCG
 121  CTGTGGGGCC CTTCCAGAGA AATGTACTGA AAAAGTCTAC GCAATGTCTG GGATTTGCTA
 181  AACAATACCT GGAAAGCAGA CAGGTTTTTT TGCCATTCCT CCAGGACATC CACCATAAGG
 241  AAAGGAGACC CTGGACCAAC ATTCTCTAAG ATGTTTATAT GGACCAGTGG CCGGACCTCT
 301  TCATCTTATA GACATGATGA AAAAAGAAAT ATTTACCAGA AAATCAGGGA CCATGACCTC
 361  CTGGACAAAA GGAAAACAGT CACAGCACTG AAGGCAGGAG AGGACCGAGC TATTCTCCTG
 421  GGACTGGCTA TGATGGTGTG CTCCATCATG ATGTATTTTC TGCTGGGAAT CACACTCCTG
 481  CGCTCATACA TGCAGAGCGT GTGGACCGAA GAGTCTCAAT GCACCTTGCT GAATGCGTCC
 541  ATCACGGAAA CATTTAACTG CTCCTTCAGC TGTGGTCCAG ACTGCTGGAA ACTTTCTCAG
 601  TACCCCTGCC TCCAGGTGTA CGTTAACCTG ACTTCTTCCG GGGAAAAGCT CCTCCTCTAC
 661  CACACAGAAG AGACAATAAA AATCAATCAG AAGTGCTCCT ATATACCTAA ATGTGGAAAA
 721  AATTTTGAAG AATCCATGTC CCTGGTGAAT GTTGTCATGG AAAACTTCAG GAAGTATCAA
 781  CACTTCTCCT GCTATTCTGA CCCAGAAGGA AACCAGAAGA GTGTTATCCT AACCAAACTC
 841  TACAGTTCCA ACGTGCTGTT CCATTCACTC TTCTGGCCAA CCTGTATGAT GGCTGGGGGT
 901  GTGGCAATTG TTGCCATGGT GAAACTTACA CAGTACCTCT CCCTACTATG TGAGAGGATC
 961  CAACGGATCA ATAGATAAAT GCAAAAATGG ATAAAATAAT TTTTGTTAAA GCTCAAATAC
1021  TGTTTTCTTT CATTCTTCAC CAAAGAACCT TAAGTTTGTA ACGTGCAGTC TGTTATGAGT
1081  TCCCTAATAT ATTCTTATAT GTAGAGCAAT AATGCAAAAG CTGTTCTATA TGCAAACATG
1141  ATGTCTTTAT TATTCAGGAG AATAAATAAC TGTTTTGTGT TGAA
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FIG.1A

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   1  MFIWTSGRTS SSYRHDEKRN IYQKIRDHDL LDKRKTVTAL KAGEDRAILL GLAMMVCSIM
  61  MYFLLGITLL RSYMQSVWTE ESQCTLLNAS ITETFNCSFS CGPDCWKLSQ YPCLQVYVNL
 121  TSSGEKLLLY HTEETIKINQ KCSYIPKCGK NFEESMSLVN VVMENFRKYQ HFSCYSDPEG
 181  NQKSVILTKL YSSNVLFHSL FWPTCMMAGG VAIVAMVKLT QYLSLLCERI QRINR
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FIG.1B